IMPACT OF A HISTORICAL TRANSLOCATION ON POPULATIONS OF WHITE-TAILED DEER ODOCOILEUS VIRGINIANUS

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Impact of a historical translocation on populations of White-tailed deer *Odocoileus virginianus.*

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ABSTRACT

Randomly amplified polymorphic DNA (RAPD) analysis was used to study the impact of a historical translocation event on populations of white-tailed deer in Georgia. The technique may also serve as a model by which management practices for other rare species can be studied. Populations from an original translocation source in Wisconsin, another Wisconsin population, and three populations in Georgia were sampled during management hunts. RAPD PCR was used to produce a genetic fingerprint for individuals of each population. Resulting band data were analyzed using PHYLIP genetic analysis software. A consensus tree of 100 bootstrapped replicates showed significant similarity between Georgia populations that received translocated deer and the original Wisconsin source populations. This indicated historical genetic impact on a local scale; however, other "native" Georgia populations which did not directly receive translocated individuals showed no evidence of genetic impact. Impact of the translocation was detected in local deer populations as a significant difference between Georgia "native" populations and Georgia populations that received translocated individuals. Low gene flow due to white-tailed deer dispersal behavior is likely to reduce the regional impact of translocations to a local scale.

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I. Introduction:

By the early twentieth century, many historically abundant wildlife species were at critically low population levels. Even the now ubiquitous white-tailed deer population was only 1-2% of its pre-Columbian density before European settlement of North America (Wing, 1965; Noble 1966). Most early efforts to reestablish rare species focused on translocation and reintroduction as strategies to create stable metapopulations. Translocation strategies continue to be in widespread use but their success is uncertain and methods are often based on more conjecture than scientific evidence (Griffith et al. 1989; Falk and Olwell, 1992; Mistretta, 1994; Lomolino and Channell, 1995; Doremus, 1999). However, using molecular tools it is now possible to study the evolutionary history of a species as well as gene flow and genetic diversity within a particular population (Mistretta, 1994). The new techniques give researchers more precise indicators of translocation success and allow substantiation of methodologies for future planning and implementation of wildlife management programs or recovery plans for endangered species.

Effective translocations and stocking efforts require extensive study and logistics, often involving not just target species but also various political, biological, and social issues (Tear and Forester, 1992; Falk and Olwell, 1992; Mistretta, 1994; Lomolino and Channell, 1995). Species management through translocation and restocking involves a complex mix of strategic issues. For example, should efforts be concentrated on maintaining currently viable populations or on those already suffering from inbreeding depression? In addition, no general school of thought exists for translocating several

small populations versus a single large translocation. Each case should be studied independently to determine the best strategy (Lomolino and Channell, 1995).

Population collapse has been studied using the island biogeographic model, which considers isolated populations as insular in their genetic behavior. It was observed that many isolated populations collapse in the center of their range and persist longest on habitat peripheries. While somewhat counterintuitive, the implications for release location can be critical (Lomilino and Channell, 1995). Finally, in any reintroduction effort, monitoring of both source and translocated populations should be continued long-term to assess the success of the project with a focus on changing whatever conditions led to population decline initially (Falk and Olwell, 1992). Lubow (1996) used mathematical modeling to optimize translocations based on population size of both origin and destination populations. Species survival may then be predicted as a function of the number of individuals moved, size of preserve and growth rate. In many cases, translocation can be an inexpensive last hope for many species previously written off as hopeless.

At current population levels, white-tailed deer afford a practical opportunity to study gene flow and genetic interaction between native and translocated populations. White-tailed deer are plentiful and samples are easy to obtain. There is little complication in sacrificing or working with the animals, as recreational and management hunts are common.

Dispersal behavior in white-tailed deer is particularly relevant to restocking efforts. Deer establish home ranges, which vary both spatially and temporally. For example, in a molecular study using beta-hemoglobin and several other proteins,

genetically similar deer populations did not correspond to traditional management boundaries or topographic barriers as expected. Instead, genetic differences between populations reflected annual movement of deer and localized allelic differences. Gene flow, which occurs through dispersal of individuals between genetically distinct populations, is primarily due to the natural dispersal of first year male bucks. Of dispersing individuals, 64%-80% tend to be yearling males, while less than 20% are young does. This dispersal behavior may be caused by sexual competition for mates (Marchinton and Hirth, 1984; Nelson, 1993).

Smith et al. (1984) recommended that deer populations be viewed as a series of small subpopulations with varying allele frequencies over short times and distances. Likewise, Scribner et al. (1997) found large fluctuation of genetic subpopulations over time and distance. Population changes can be genetically or environmentally influenced, and generally, high levels of heterozygosity in a population correlate to an overall increase in fitness (Smith et al. 1984).

In addition to protein level research, there have been numerous recent studies, using polymerase chain reaction (PCR) to conduct taxonomic and population genetic analyses on deer translocations. Ellsworth et al. (1994a) used mitochondrial DNA from deer and restriction enzymes to study gene flow and dispersal as well as overall genetic impact of translocated deer in the southeast. This study found that regional history dictated genetic variation. Deer populations in the south are older, owing to less recent disturbance from glaciation, unlike the northern subspecies. Thus, southern deer have higher genetic variation than their northern counterparts, resulting from a longer time for genetic divergence to occur. Fragmentation and isolation of southern populations has

also led to differences in mtDNA. Due to the matriarchal structure of deer populations and dispersal primarily through young males, mtDNA tends to remain stable within populations in spite of human influence and translocations (Ellsworth et al. 1994a; Scribner et al. 1997). If translocations had an effect on recipient deer herds, it would be expected that genetic variation in a herd receiving relocated individuals would reflect the introduction of the new genotypes.

This was not the case in a DNA study by Ellsworth et al. (1994a, 1994b), which concluded that the recovery of deer herds in the southeast resulted from natural re-establishment and management of native deer rather than direct offspring or hybrids from translocations. Leberg et al. (1994) in a similar study using protein allozymes in deer and wild turkeys found that subpopulations receiving translocated individuals were more genetically similar than subpopulations not affected by translocations. This would indicate successful reproduction and gene flow from translocated deer and turkey. The number of individuals translocated was not a significant determinant of genetic impact. Even a few non-native individuals were found to have an impact on local subpopulations. Overall, translocations were seen to have an impact on specific subpopulations where they were released but not on overall local populations due to low gene dispersal over a long distance.

Conclusions from the two studies seemingly contradict one another. However, the two research groups published a third joint paper, in which they reanalyzed the data from both studies and drew more unified conclusions. Ellsworth et al. (1994a, 1994b) found minimal regional effects from translocations. Neighboring populations were more genetically similar to one another due to dispersal between local populations.

Mitochondrial DNA and isozyme variation was similar suggesting common evolutionary influences for both types of markers. Finally, mtDNA variation in deer from around the southeast is similar to that of other related taxa. The similar mitochondrial genetic variation can indicate a common historical background across different species and would indicate a natural mutation rate rather than any impact from translocation. The discrepancy with Leberg et al. (1994) arose from sample choice and type of analyses used for the study rather than any fundamental disagreement in data (Leberg and Ellsworth, 1999). Both regional and long distance translocations seem to have a localized impact, but due to low female dispersal and site philopatry there is little gene dispersal to neighboring populations. From a management perspective, restocking may be beneficial but the genetic impact of this strategy is only felt at the subpopulations.

Small but genetically distinct local populations may be overwhelmed by an indiscriminant translocation event. Dispersal is unlikely to restore the genetic diversity lost when the source population overwhelms the recipient population, making the local area more genetically homogenous. Dispersal is also less likely to maintain diversity in endangered populations, which are more likely to suffer reproductive isolation (Leberg and Ellsworth, 1999). Also, if translocations take place over great distances, additional problems may arise from low survival rates of translocated individuals placed in areas for which they are not adapted (Leberg and Ellsworth, 1999; Day et al. 1999). Ideally, initiation of any restoration project would therefore involve conservation planning to re-establish native remnant populations. Detailed genetic studies of both source and destination populations should occur before any restocking to avoid disruption of the genetic structure at the local level (Leberg and Ellsworth, 1999; Mistretta, 1994).

Leberg and Ellsworth (1999) suggested that future studies should investigate the rate and success of gene introgression from translocated deer into native herds. Additionally, there has been no direct comparison of genetic diversity from northern and southern populations. My research was designed to address both areas using the molecular technique of randomly amplified plymorphic DNA (RAPD) PCR to study white-tailed deer translocated from Wisconsin to Georgia.

Benefits involved with RAPD analysis are many, and application of this process to the study of molecular taxonomy and population genetics has been fruitful for a range of genetic analyses including taxonomic differentiation, gene flow and hybridization, paternity and kinship, and genetic mapping (Hadrys et al., 1992; Williams et al., 1993; Lynch and Milligan, 1994; Comincini et al., 1996; Kostia et al., 1996). RAPD PCR uses arbitrary, short oligonucleotide primers to produce diagnostic polymorphisms for fingerprinting genomic DNA. The RAPD technique arose as an alternative to expensive and time-consuming restriction fragment length polymorphism (RFLP) studies and uses primers that are able to amplify DNA fragments with good reproducibility. Bands are visualized, after agarose gel electrophoresis, using ethidium bromide and ultraviolet light. Markers can detect even single base pair mutations in template DNA, as well as deletions in the primer binding site or insertions between binding sites which elongate the binding template strand but allow it to remain short enough to amplify (Williams et al., 1990). These short arbitrary primers are able to provide a characteristic fingerprint for any genome, and protocols for their use are defined and supported by numerous studies (Williams et al., 1990; Welsh and McClelland, 1990; Caetano-Anolles et al. 1991; Williams et al., 1993).

Unlike previously used codominant genetic markers, RAPD fragments are inherited in a simple dominant-recessive fashion, which can only detect presence or absence of an allele at a given locus. Previously used polymorphisms codominant inheritance can detect heterozygotes. However, RAPD's resolve many loci for a given individual simultaneously. This differs from RFLP's also, which detect few loci but are able to find multiple alleles at each locus (Black, 1993; Williams et al., 1993).

There is an array of variables involved in RAPD analysis, which must be optimized to avoid discrepancies in data. Their variables make RAPD analysis inherently sensitive to perturbations or imprecise technique. The specificity of results to polymerase and thermocycler brands used also make comparison of data between labs or between studies difficult. Meunier and Grimont (1993) recommended extreme caution when assuming gel data in its raw form can be used by another research facility. Rather, they recommended that RAPD gels should not be cataloged in a library or database for future studies. The technique could, however, be used independently as a tool for an individual study.

RAPD amplification conditions typically use lower annealing temperatures than would be used for a primer with known sequence, and cycle profiles must be determined empirically to obtain consistent results. Williams et al. (1993) recommended the following general profile:

25μL initial reaction volume
94°C 1min. – denaturation of template DNA
36°C 1min. – primer annealing to ss template DNA
72°C 2min. – elongation of regions flanked by annealing primers
X 45 cycles

The products of this reaction are variable depending on the concentration of genomic DNA used, and, as such, the isolation protocol should be uniform throughout the study. Isolation products may also be quantified using a spectrophotometer to ensure uniformity throughout the sample. Generally, RAPD products reproduce well using as little as 1μg of template DNA. Less than this may yield irreproducible products, while too great a quantity of template DNA results in low band clarity and smearing when stained (Williams et al., 1993).

RAPD analysis requires a number of assumptions. First, interpretation of gel bands must be unambiguous. The assumption is made that no two markers will co-migrate to the same position on a gel; thus, each band represents a single locus and no experimental errors are made during scoring. Second, each locus is treated as a two-allele system scored as either present or absent. This scoring system is based on the dominance of RAPD markers, which are scored recessive if a particular band is not amplified. Reasons behind band absence may be due to primer site deletion or base insertions, making the fragment unamplifiable. The resulting alleles are assumed to follow Hardy-Weinberg models for sexual diploid populations where (p) is defined as the proportion of homozygous dominant alleles in a population and (q) is the proportion of homozygous recessives and ($q = 1 - p$). Frequency of markers in a population can be accurately estimated using RAPD primers. Markers should be rare enough that they do not occur commonly in each population. Gene diversity is also detectable as RAPD's typically amplify non-coding DNA. Lynch and Milligan (1994) used RAPD's to calculate genetic distance and relatedness between populations, as well as, genetic diversity. The same study recommended using a greater number of loci for calculations

and larger sample sizes, with the same number of samples for each study population. This compensates for the loss in accuracy from RAPD compared to those techniques using codominant markers.

Analysis of RAPD data may be accomplished using any of several computer software packages. Felsenstein (2000) developed the PHYLIP 3.6a package, which is in wide use for RAPD data, and many other molecular techniques. RAPDistance 1.04, a RAPD-specific package, and PAUP, a multi-use package similar to PHYLIP are also used widely (Armstrong et al., 1996; Swofford, 2000). These packages allow bootstrapping, calculation of genetic distance matrices and synthesis of phenetic trees. Trees can be drawn to scale using a variety of software as well, including Tree View 1.5.2 (Page, 1998).

My study, used RAPD PCR techniques is an attempt to determine the effects of a historical translocation of white-tailed deer. During the first half of the twentieth century, the white-tailed deer populations were decimated in the southeast due to over hunting and poor management. Throughout the region, aggressive restocking efforts were made in an attempt to augment remnant native herds. In 1962, 639 deer of both genders were translocated from central Wisconsin to areas around central-west Georgia (Jeffries, 1975). Source location for many restocked individuals was Sandhill Game Farm in Wood County, Wisconsin owned by Wallace Grange. In 1963, the farm was bought by the State of Wisconsin and renamed Sandhill Wildlife Area (Wayne Hall and Mark Randall; personal communication). The translocated deer, *Odocoileus virginianus* subspecies *borialis,* are a northern subspecies. Typically the northern type are

morphologically larger, heavier deer in comparison with the native southern subspecies *O. virginianus* subspecies *virginianus*.

One destination for translocated deer was Harris County, inside what is now Callaway Gardens (see figure 1). The Gardens (G) sample population was taken from inside an area enclosed for the last twelve years by a deer resistant fence. Presumably, the fencing has somewhat restricted gene flow, although damage and failure to close gates may have resulted in some migration of deer into and out of the area. A second sample group from the same area is called Harris (H), with individuals sampled from outside the fenced enclosure but still in the immediate area.

Northern deer subspecies translocated from Wisconsin to Harris Co. Georgia also display greater seasonal migratory behavior than do southern subspecies. This difference in temporal dispersal pattern has direct implications for gene flow between translocated and native populations in the south. While southern subspecies tend to display more fixed ranges, northern deer may travel more and disperse among local southern populations. Increased gene flow into remnant native populations would be expected if translocated northern populations were moving over greater distances and interacting with native herds (see figure 2). For this reason, a third population of deer from Marion County (M) served as a control (figure 1). This population did not receive translocated northern deer and it is approximately 50 miles from the Harris County experimental populations (see figure 1).

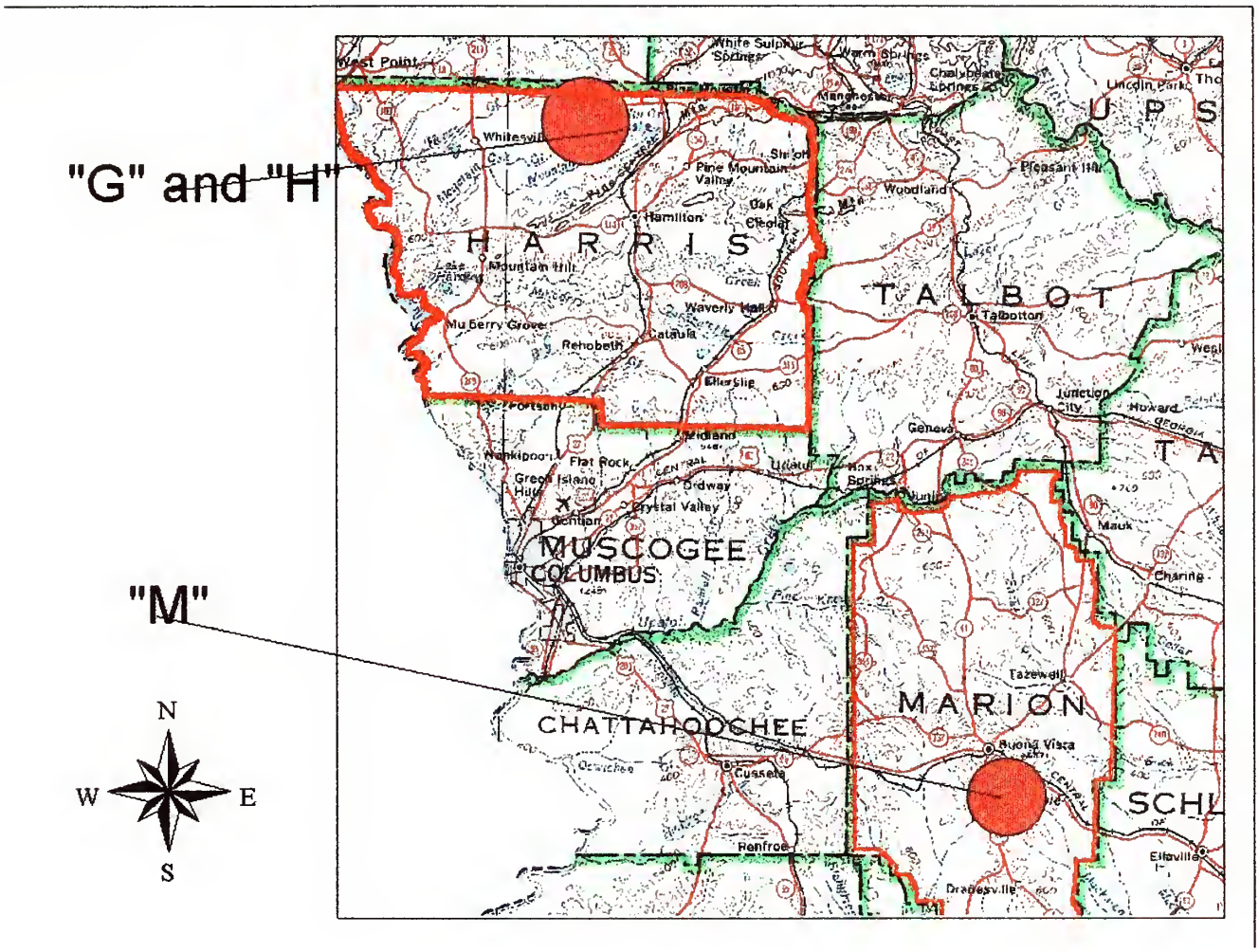


Figure 1.
Population locations in Marion County (M) and Harris County (G and H).

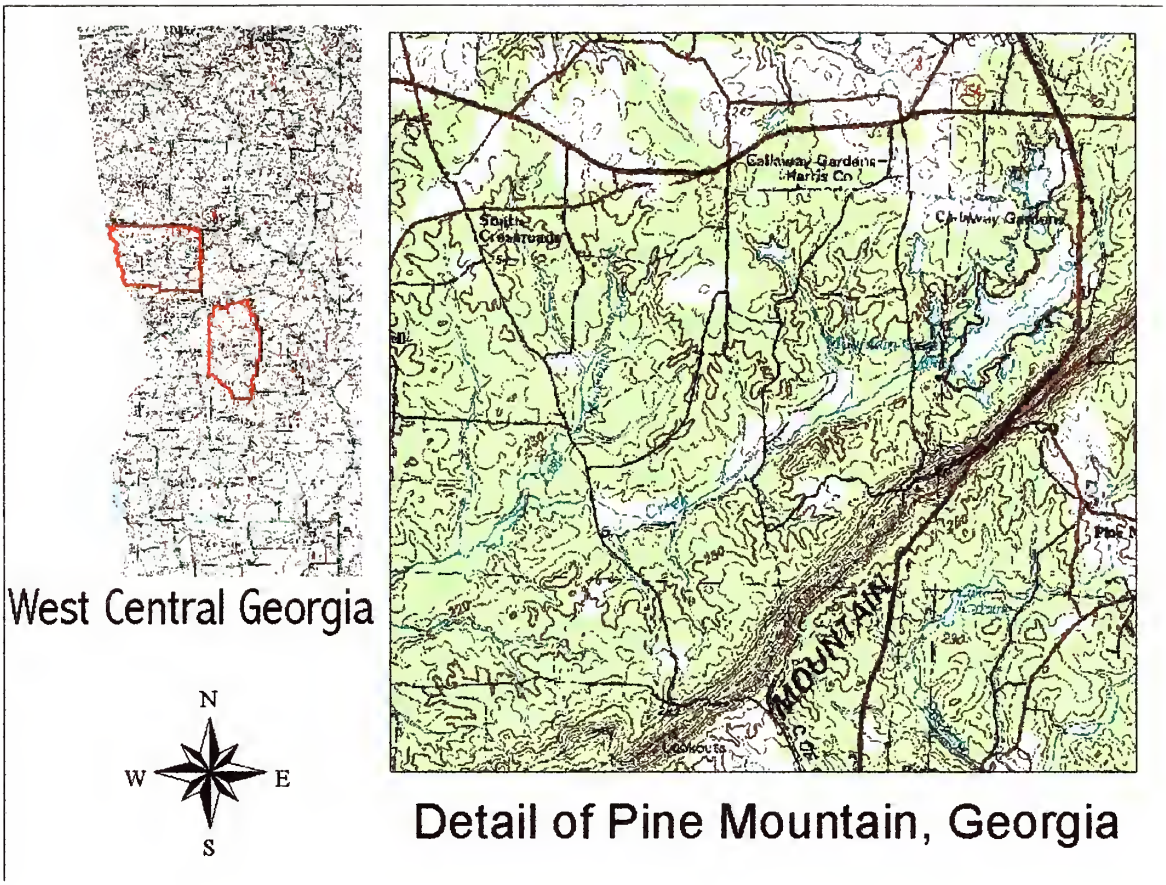


Figure 2.
Detail of Gardens and Harris deer source location.

From a broader perspective, the successful recovery of southern deer populations from remnant, isolated native populations may be used as a model for future recovery plans for currently endangered ungulate populations. The success or failure of historical translocations and the genetics involved may shed light on the current use of these strategies and shape management and recovery plans. Today's molecular tools enable the direct observation of gene flow and are recommended to be part of any endangered species recovery plan (Mistretta, 1994; Lubow, 1996). The effectiveness of RAPD PCR for quick and inexpensive assessment of population restocking efforts could be used to examine other historical species management techniques for rare or endangered species.

It may also be an effective method to identify the best source and recipient populations in the field during current or future translocation efforts.

Two hypotheses are used to steer this study. In one case, the translocation event would be regarded, in hindsight, as a success in reestablishing local native herds, while the opposite would indicate the translocation was unnecessary and had no significant genetic effect on local herds.

> H_1 - Deer populations in Harris Co., which were restocked with translocated individuals from Wisconsin, are more genetically similar to a local native population than source populations of restocked individuals.
>
> H_2 - Deer populations in Harris Co., which were restocked with translocated individuals from Wisconsin, are more genetically similar to source populations than to a local native population.

II. Methods:

A. Tissue Handling and Storage:

All samples were collected between February and November 1999. Muscle, blood, and liver samples were obtained from 100 individuals inside a partially enclosed area of Callaway Gardens (G), which received translocated deer from Sandhill, Wisconsin in 1962. Muscle, blood, and liver were also obtained from 50 individuals in surrounding Harris County (H) and 10 individuals from Marion County (M) used as an outgroup. Muscle and liver samples were obtained from 50 individuals inside the Sandhill Wildlife Management Area (S) in central Wisconsin (original source location), and approximately 50 muscle samples were obtained from game check stations across the northern peninsula of Wisconsin (W). Tissue was taken from individuals of both genders and all age classes at various times of year. Muscle and liver samples were stored in sterile vials and fresh-frozen to negative 80°C. Blood samples were stored in tubes

treated with heparin as an anticoagulant and separated into cellular and plasma fractions before storage at negative 80°C. Tissue samples were kept on ice for several hours in the field before freezing. Each sample was labeled with a number and letter identifying from which population it was gathered (i.e.- 45H, 67G). Muscle samples were taken from the abdominal region after the animals were sacrificed either for herd management or recreation hunts.

B. DNA Isolation Procedure:

Muscle tissue comprises the bulk of samples from each location and so was used for isolation of genetic material. DNA was isolated using DNeasy™ tissue isolation kits (Qiagen catalog number 69504). Each kit uses tabletop centrifuge tubes and proprietary buffer solutions to isolate known quantities of DNA from animal tissue (Figure 3). The DNeasy™ system uses silica gel membranes to collect DNA without organic extraction or ethanol precipitation (Figure 4.). Differences in template DNA isolation affect primer annealing during PCR and can be particularly problematic when ethanol precipitation is used during isolation. Treatment with RNase must also be uniform throughout the study to avoid inconsistencies. Cells are lysed and buffers, which optimize conditions for DNA binding to the silica membrane, are applied. After centrifugation and selective binding of DNA to the membrane, contaminants are removed in two wash steps before DNA is eluted for final storage and use. Typical expected yield for this procedure is 15-20µg of DNA per sample. DNA fragment size ranges up to 50kb but 30kb segments dominate (Qiagen, 1999). This DNA isolation procedure was chosen based on purity and

consistency of resulting DNA for PCR and on time savings compared to other methods (Figure 5).

Figure 3.
Schematic of the DNeasy™ DNA isolation procedure. (Figure taken from Qiagen, Inc.)

Figure 4.
DNeasy™ spin column used in isolation procedure. (Figure taken from Qiagen, Inc.)

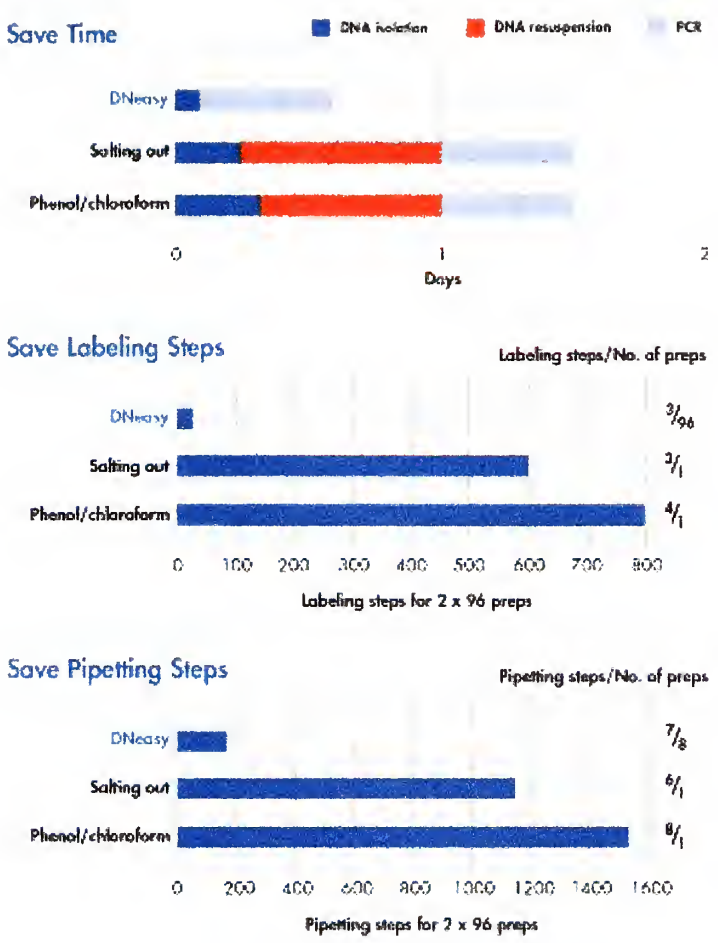


Figure 5.
Comparison chart of various isolation steps (figure taken from Qiagen, Inc.).

Muscle samples were thawed at room temperature and small sections were removed. Care was taken to prevent cross contamination between different samples. Muscle cells are multinucleate and are interspersed with fibrous connective tissue making lysis more difficult. To avoid clogging the silica isolation membranes a maximum of 12-15mg of tissue was used.

1. Each tissue sample was placed in a sterile 1.5ml microcentrifuge tube. Tissue was lysed using 20µl of Proteinase K and 180µl of proprietary buffer ATL added to each tube. Samples were incubated at 55°C in a shaking water bath overnight.

2. After lysis, samples were treated with 4µl of RNase to remove any residual RNA, which could interfere with PCR.
3. 200µl of proprietary buffer (AL) was added to each sample and incubated for 10min. at 70°C on an MJ Research PTC-100 thermal cycler.
4. 200µl of absolute ethyl alcohol was then added to each tube.
5. The entire mixture was then pipetted into a DNeasy™ spin column and centrifuged for 1min. Column flow through was discarded.
6. The column was placed in a new collection tube and 500µl of proprietary buffer (AW1) was added and centrifuged for 1min. Flow through was discarded.
7. Step 6 was repeated using proprietary buffer (AW2) and centrifuged for 3min.
8. Isolation columns were placed in final collection tubes and 200µl of proprietary buffer (AE) was placed in each tube. Tubes were centrifuged for 1min. This last step was repeated and the collection tube now containing eluted DNA was labeled and stored at -20°C.

Initially, eluted DNA samples were then quantified using a Hoefer Dyna-Quant™ spectrophotometer. However, the confirmation proved difficult and was abandoned due to unexplained fluctuations in control samples and calibration of the spectrophotometer. It was assumed that expected yield, as standardized by Qiagen Inc., was approximately correct for the actual amount of DNA isolated from each sample.

C. Polymerase Chain Reaction (PCR):

The RAPD PCR technique was used for this study. Template DNA was obtained using the procedure above. RAPD primers were obtained from Operon Technologies Inc. in 10-mer kits. Primer sequences were randomly chosen by Operon Inc. All primers are 10 base pairs in length with 60-70% G+C content and are designed with non-complementary ends to avoid self-annealing. Primers were lyophilized for storage and resuspended in TE buffer at pH 8.0. Initial scans to optimize primer/template systems were done using Operon kits OPA, OPC, OPD, OPE, and OPT. Each kit contained 20 different sequences.

Klein-Lankhorst et al. (1991) found that by changing PCR conditions or by using different DNA isolation procedures, different banding patterns were produced. However, unique fragments were still amplified for each primer, and even single base changes were detected. This inherent variability in product may result from PCR inconsistencies, template to primer concentration ratio and annealing temperature, all of which require consistent protocols and experimental design throughout a study. Failure to maintain consistent techniques or reaction conditions results in irreproducible results and greater error in analysis of data (Perez et al., 1998).

To increase the reproducibility and usefulness of results a variety of PCR conditions were attempted before the system was optimized. Initial scans using various primer, template, and buffer concentrations, and three different types of DNA polymerase were tested. For initial scans, DNA from three deer in the Callaway Gardens (G) population (206G, 209G, 79G) were used as template for all primers in each of the five Operon Technologies kits listed above. Primers yielding two or more strong bands when

resolved on 1.4% agarose gels were then chosen as possibilities and run again, using an array of DNA from four sample populations (S11, S15, W10, W11, 200G, 170G, 48H, 59H). This was done to determine which amplification products showed variation across all experimental populations. Comparison of gels for bright, clear, multiple bands, which were also somewhat variable among sample populations yielded a final selection of four primers as optimum for this project (table 1.). DNA from 16 individuals was isolated from each population with the exception of the Marion County out-group of which only eight individuals were used.

Table 1.
Operon Technologies, Inc. primer sequences used for final analysis.

Operon RAPD Primer	Primer Sequence
OPC-6	GAACGGACTC
OPC-8	TGGACCGGTG
OPC-9	CTCACCGTCC
OPT-4	CACAGAGGGA

The DNA polymerase chosen for optimal amplification products was Taq DNApol from Qiagen Taq PCR Master Mix Kit™ (Qiagen catalog number 203443). This product is a recombinant 94-kDa polymerase isolated from *Thermus aquaticus* expressed in *E.coli.* The polymerase extends DNA at 2-4kb/min at 72°C and has a half-life of 10min. at 97°C. This Taq has 5'-3' exonuclease activity allowing greatest reproducibility in amplified fragments. Taq was shipped and stored suspended in a 2X concentrated master mix containing proprietary PCR buffer, $MgCl_2$, and 400μm of each dNTP (see figure 6).

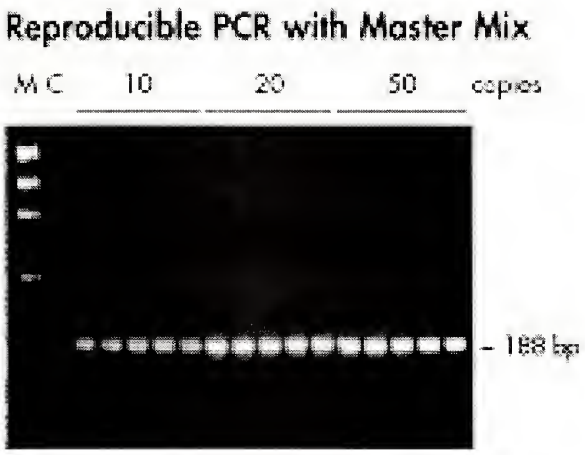


Figure 6.
PCR reproducibility comparison gel (figure taken from Qiagen, Inc.).

All PCR preparations were carried out at room temperature inside a sterile fume hood, using aseptic technique to prevent contamination. Materials, equipment, and lab surfaces were sterilized by autoclaving and/or ultraviolet light before use. PCR ingredients were stored at -20°C and thawed at room temperature. 12.5µl of Taq Master Mix was placed in sterile reaction tubes along with 10.5µl of sterile deionized water. To this, 1µl of sample DNA template (15-20µg/200µl) and 1µl of primer DNA (25pmol/µl) were added. This PCR mixture was then pulse vortexed to eliminate local variation in reactant concentration before thermocycling.

Amplification was accomplished using an MJ Research PTC-100 thermal cycler equipped with a thermocoupled, heated lid. A heated cover removed the need for mineral oil overlays in the PCR reaction tubes. PCR thermal cycling used a cold start. Transition times for thermal cycling steps were ramped from one to another as quickly as allowed by the cycler. Cycling profile was as recommended by Williams et al. (1990) (table 2).

Table 2.
PCR thermal amplification profile.

Melting Phase	94 C	1min.
Annealing Phase	40 C	1min.
Elongation Phase	72 C	2min.
X 45 cycles		
Hold Phase	0 C	10-20 hr.

After amplification, PCR products were stored in original reaction tubes at -20°C for various lengths of time before electrophoresis.

D. Agarose Gel Separation:

The gel media used Bio-Rad™ Analytical Grade Agarose (catalog number 162-0125) melted in a standard microwave oven in glass Erlenmeyer flasks. Agarose was diluted in 1X TBE buffer solution prepared to 5X concentration in 1L alloquots (54g Tris Base, 27.5g Boric Acid, 20ml 0.5M EDTA at pH 8.0, up to 1L with deionized water).

Initial gels to confirm the presence of isolated DNA used 0.7% Agarose prepared using 50ml gel molds with small wells (0.35g agarose, 50ml TBE buffer, 2.5µl ethidium bromide). Loading assay for the 50ml gels used 5µl of sample DNA, 13µl TBE buffer, and 2µl loading dye. Gels were run using an external electrical power pack for 2 hours at 80mA.

Larger 1.4% gels were used to separate PCR material for visualization and analysis. These 200ml gels were prepared using 2.8g agarose, 200ml TBE, 10µl ethidium bromide with 40µl gel wells. Loading assay for the analysis gels used 12.5µl

PCR sample, 22.5µl TBE, and 5µl loading dye (0.5µg/ml). These gels were run at 160mA for 4 hours. Eight samples of PCR product were run on each gel along with two control samples (G-80, S-19), a 1000bp ladder, and a 100bp ladder. Ladders were prepared by Amresco™ (code number K181 and K180 respectively). A control sample, containing each PCR reactants except template DNA, was also included on each gel. These controls were prepared simultaneously with each batch of PCR product to rule out contaminants as contributors to any amplification products.

After each electrophoresis run, gels were removed from their trays, placed on an ultraviolet light box with a metric scale, and photographed using a Polaroid™ camera equipped with a light proof sleeve and red filter. Exposure was from 6-8 seconds depending on band brightness and photo resolution. F-stop was set for F-16. Photographs were then cataloged and stored before gel disposal.

E. Band Scoring:

Before scoring, each gel was "calibrated" using migration distance, measured by caliper, of the known 100 base pair ladder. Migration distance plots of each gel were created using semi-log scale with migration distance on the x-axis and known molecular weight ladder on the y-axis (figure 7). Semi-log scale appropriately reflects the nature of DNA fragment migration through the gel.

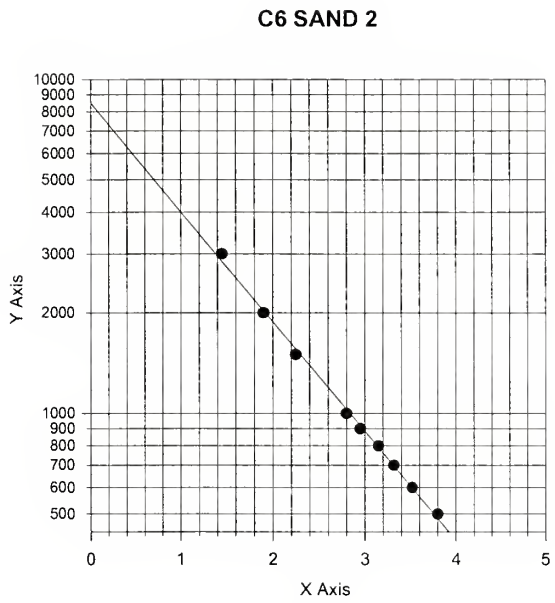


Figure 7.
Gel ladder migration plot.

Subsequent band scoring proceeded, using ladder migration plots to fix the known rate of migration for each gel. Unknown bands were then compared to the linear ladder plot to determine approximate fragment length. In this way, variation in fragment length could be determined accurately for scoring between bands of similar molecular weight. There were two discrete strategies used to score band data. The second technique was ultimately used for analysis based on overall accuracy of data.

In the initial scheme, gels for each primer and population were examined visually to identify the best bands for subsequent scoring. Bands were identified based on brightness, which was pivotal for reliable scoring, and consistency of reproduction. The identity of each band was determined using the calibration technique described above. In this way, 4 – 8 bands were identified in each primer set for scoring. Individuals were recorded as either showing a given band or not.

This approach proved problematic for dealing with bands that had similar migration distances for different samples. Discerning bands that differed by only a few base pairs involved too much subjectivity for scientific comfort.

In an attempt to resolve this scoring difficulty, a second scoring scheme was used for the final analysis. This approach involved scoring each individual for all bands visible within a range of molecular weights. For instance, between 3000 and 600 base pairs. As scoring progressed it became easier to resolve one band from another for a given primer group. This method is similar to that used to score alleles in starch gel electrophoresis but scoring each band individually rather than alleles.

RAPD markers are inherited in a dominant fashion and as such are di-allelic. Presence or absence of a given band is treated as one of two alleles at a particular locus identified by band location. Based on this inheritance, each scored band for all individuals in a population was assigned a value of (1) for the presence of a marker band or (0) for the absence of the same marker. Due to requirements of analysis software and to avoid unnecessary "noise," individual deer with amplification failures for any of the four primers were not considered for analysis. This had the effect of removing 17 individuals from analysis but ensured that the same individual DNA samples were used across all four primers.

Scored data derived using this method, the second scoring scheme, and the calibration mentioned above, may be found in Appendix I. It should be noted that, although some band weights are the same for different primer sets, they should not be misinterpreted as the same fragment of amplified DNA. Rather, bands from different

primers that have the same molecular weights simply reflect a similar length fragment but would have a different base pair sequence.

F. Data Analysis:

Raw band data was analyzed using the PHYLIP 3.6a phylogenetic inference software package (Felsenstein, 2000). This software uses the MS Windows™ platform and provides a variety of analysis tools for molecular and population genetics. The programs described below are contained within the PHYLIP package.

Before analysis, the raw data was resampled using SEQBOOT to produce a set of replicate data sets from the original set (Felsenstein, 1985). The SEQBOOT program samples *n* characters in the data, randomly, with replacement. The resulting dataset has the same total number of individuals as the initial dataset, with some individuals being duplicated and some left out (table 3). One of the primary assumptions for bootstrap analysis is independence of mutation resulting in presence or absence of a fragment. With RAPD fragments, some bands may be influenced by the presence or absence of other bands as shown by Perez et al. (1998), calling the assumption of independent mutation into question and casting doubt on validity of bootstrap analysis.

Table 3.
Sample input data file for SEQBOOT showing correct formatting of data for analysis.

```
 5  6
Alpha    AACAAC
Beta     AACCCC
Gamma    ACCAAC
Delta    CCACCA
Epsilon  CCAAAC
```

Data was bootstrap resampled 100 times using SEQBOOT. The resulting 100 replicate datasets were then used to calculate a genetic distance matrix using RESTDIST, a program designed to address restriction fragments, but which may also be used for RAPD fragments (Felsenstein, 2000). The RESTDIST matrix for this study is a modified form of the Nei and Li (1979) genetic distance method, and assumes that fragments are accidental byproducts from random mutation of the DNA nucleotide sequence (figure 8). This mutation model for fragments assumes a Jukes-Cantor (1969) model of change.

The restriction fragment distance, which is used here for RAPD fragments, is the average probability that no new primer site will occur in between the ends of a fragment, and that the ends of the fragment do not mutate. Operon Technologies™ RAPD primers are 10 base pairs long. This allows the convenient assumption, which seems valid, that a pair of 10bp primers mimics the behavior of a 20-nucleotide restriction site for the purposes of this algorithm. Resulting distance matrices are in the form of a square, one for each replicated dataset.

In order to visualize information contained in the data matrix, a tree branching diagram must be calculated. For these data, the PHYLIP program FITCH was used to create tree branching representations (Felsenstein, 2000). FITCH uses the Fitch-Margoliash least squares method for tree calculation based on data in a pairwise distance matrix. This method assumes each distance is independent of the others in the matrix, and that distance between each pair of individuals is from a distribution in which the expected amount of mutation is the sum of values from one branch tip to the other (figure 9). Both these assumptions are somewhat dubious, as independence may not hold for most types of data including RAPD fragments. The FITCH algorithm computes the sum

of squares of the distance between two individuals and then minimizes that value by iterative calculation in order to find the shortest branch length for the resulting tree.

$$f = \frac{Q^{2s}}{2 - Q^{s}}$$

where: Q = probability of no mutation

s = mutation site length

Figure 8.
Modified form of the Nei and Li equation used by the RESTDIST program in PHYLIP (Felsenstein, 2000).

sum of squares =

$$\sum_i \sum_j = \frac{n_{ij}\left(D_{ij} - d_{ij}\right)^2}{D_{ij}^{\,p}}$$

where:

D = observed distance between species i and j

d = expected distance

n = number of times each distance is replicated

$p = 2$

Figure 9.
Least squares distance algorithm used by the PHYLIP program FITCH to calculate genetic distance (Felsenstein, 2000).

The output from the FITCH program is an unrooted tree, or in this case 100 unrooted trees, one for each replicate created in the bootstrap step. Branch length corresponds roughly to substitutions per base in the DNA sequence.

The final analytical step for the data involved creating one consensus tree from the 100 replicated samples (figure 10 and 11). The PHYLIP program CONSENSE accomplishes this using the extended majority rule to create a consensus tree diagram

(Felsenstein, 2000). Under this system, any set of individuals that cluster together in more than 50% of the trees calculated is placed in the final consensus tree. That is, if a group of individuals is present in more than 50 replicate trees, it is placed in the final tree. CONSENSE then adds other sets of species in order of frequency in which they appear until the entire tree is resolved. Included groups of individuals must not contradict another group that appears with greater frequency. Resulting tree branch lengths are numbered by the number of times a group appeared in replicated trees.

Tree diagrams were drawn using TreeView 1.6.1, which reads tree files from a variety of programs and converts the file into a modifiable, presentation format. TreeView software also enables manipulation of tree diagrams including collapse or separation of groups, choice of out-group, and type of branch diagram drawn. To avoid presenting an arbitrary representation of the data, no post-statistical (i.e. after running SEQBOOT) changes have been made. Both a rectangular tree diagram and a radial dendrogram representation are depicted in figure 10 and 11. Both trees are statistically unrooted, although square diagrams are drawn using an arbitrary rooted out-group for visualization (figure 10 and 11).

III. Results:

Figure 10 is an unrooted radial tree diagram from bootstrap resampled original RAPD fragment data. Numbers at each fork represent the number of replicate samples out of 100, in which a given group of samples clustered together. Branch length represents the confidence level of a given cluster based on number of times a group was present in the bootstrapped data. Each of the hundred replicates was weighted and the

genetic pairwise distance value multiplied by that factor to create a total final value for each individual. The first group (cluster B), clustering together 98 times, includes individuals from both translocation source populations (W, S) and recipient populations (G, H) with one individual from the native group (M7). The second cluster (cluster A), occurring 84 times was exclusively composed of Marion County (native) deer. All other clusters occur in less significant numbers with the exception of one cluster between two individuals 3W and 21H. Landry and Lapointe (1997) recommend considering bootstrap values greater than 70% significant.

Figure 11 is a phylogram of the identical data in figure 10. The outgroup for this diagram is arbitrary. Again, as in figure 10, the cluster (A) containing the "native" Marion County individuals cluster together with the Wisconsin deer 16W the next sister group. In this diagram, branch lengths roughly correspond to substitutions in the base pairs of DNA from the individuals (Felsenstein, 2000). In both diagrams the native control individual M7, clusters outside the (A) group of other native deer clustering instead with a Wisconsin deer. It should be noted that this clustering with the Wisconsin deer does not occur in a significant number of possible trees.

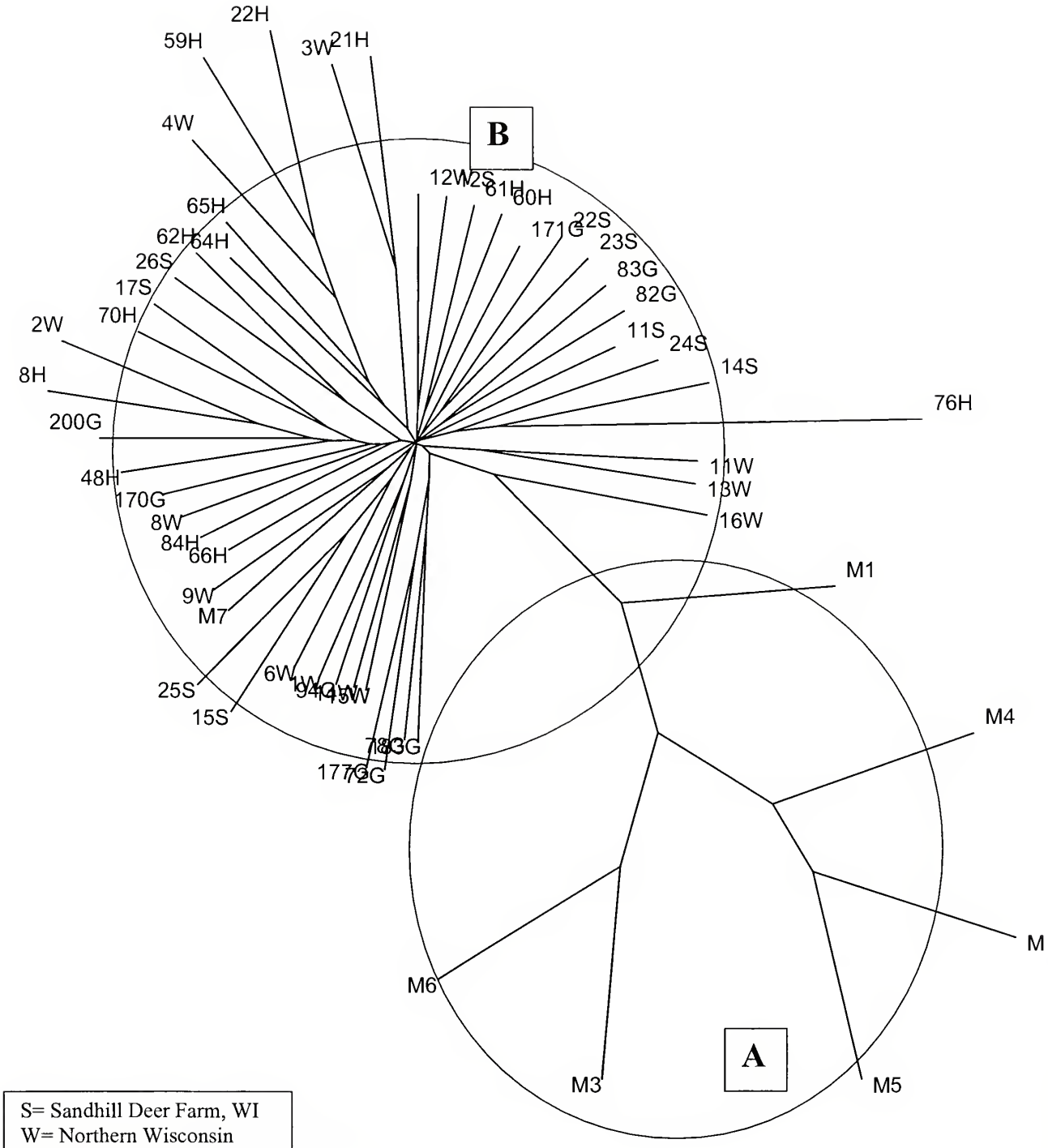


Figure 10.
Unrooted consensus radial tree diagram.

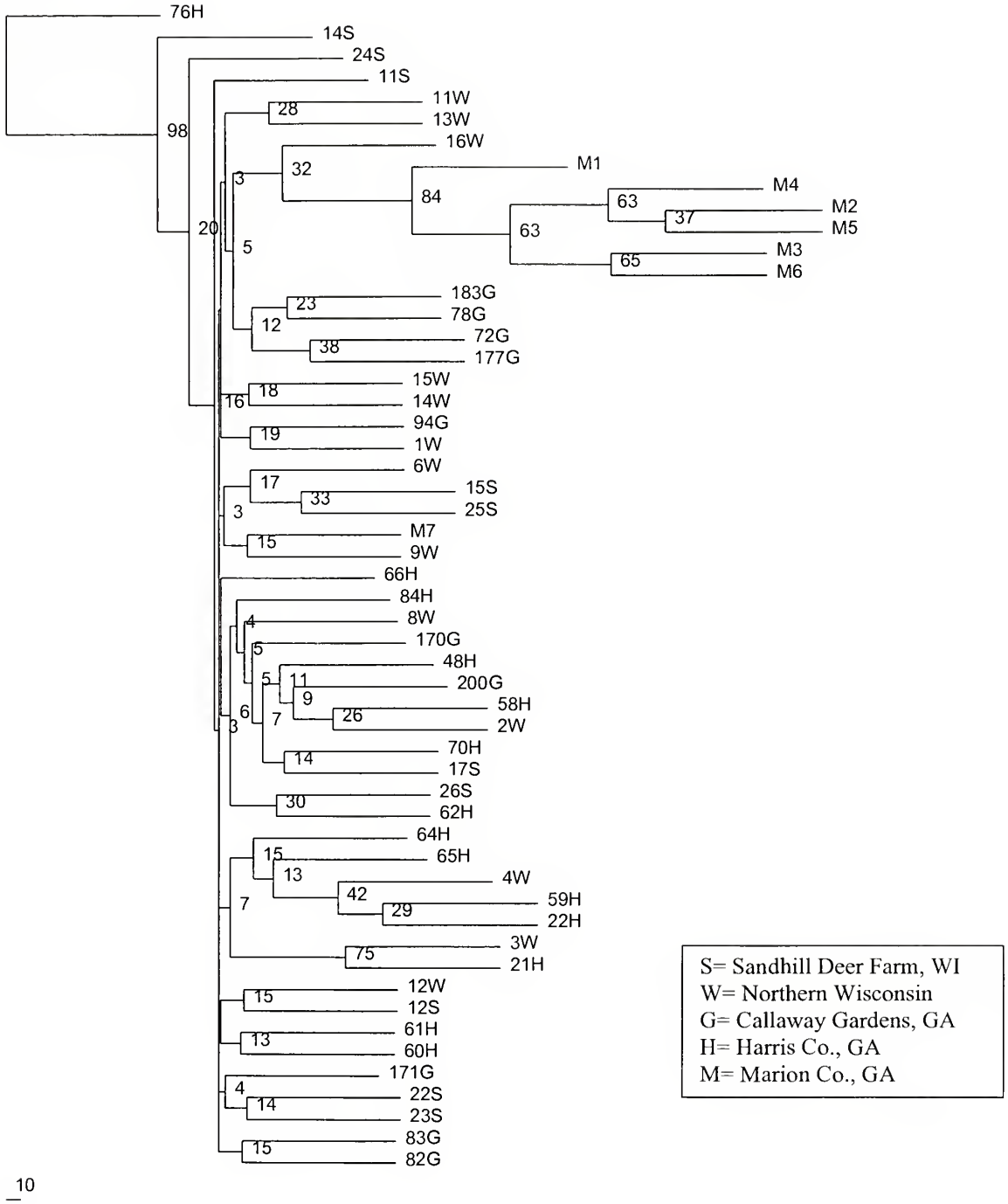


Figure 11.
Arbitrarily rooted consensus tree diagram. Branch numbers indicate the number of replicates out of 100 in which the branch occurred.

Raw band scoring data may be found in Appendix I, organized by population, with individuals in the vertical columns and fragment length for each unique fragment on the horizontal.

IV. Discussion:

If differences in genetic variation and allele frequency between the experimental populations exist, it is possible that these differences arose from the translocation event in question. If the Harris and Gardens populations are more genetically similar to the Wisconsin and Sandhill deer than to local native herds, then the translocation can be considered to have had some measurable effect. If the study herd is more similar to local populations, then the opposite would be true and non-local individuals did not make a significant reproductive contribution to present populations. Since RAPD analysis has not been used previously in this way, results may differ from those seen using protein isozymes or other methodologies.

Conservatively, it seems that the intention of the historical translocation was realized. Specifically, interaction between native deer herds and translocated individuals is present in the data, indicating that the translocation positively influenced local deer populations. While the amount of interaction cannot be well quantified using these techniques, it is supported at the immediate local level in the study populations.

The local recipient populations (G, H) and source populations (W, S) cluster together 98 times out of 100. This seems to indicate a genetic similarity between sample individuals likely due to interbreeding and hybridization of native deer populations with translocated populations. The second strong cluster, occurring 84 times in the bootstrap, was exclusively composed of Marion County (native) deer. No populations other than

Marion County are represented in the cluster, suggesting that the native population is unaffected by translocated genes. All other clusters occur with far less frequency.

However, there is little evidence to support a regional impact from this translocation effort. Tissue samples from Marion County showed significantly different genetic stock from those within the Gardens or Harris County populations. Again, an actual rate of introgression is difficult to quantify but no significant impact on the Marion County population seems to have occurred. No evidence in this study would suggest that introgression rate is constant in all geographic directions, and this lack of gene flow to Marion County may not hold true for other counties in the region.

The tight clustering of Harris County and Callaway Gardens deer from Georgia with northern deer from Sandhill and north Wisconsin indicates very little difference between the four populations. In fact, the greatest number of times a sub-cluster is found in the replicates is 38 – far below any significant cutoff. By contrast, the Marion County deer cluster together in 84 replicates, indicating a greater confidence that the two clusters are significant.

In addition to inherent uncertainty, RAPD fragments have the additional complication of fragment reproducibility. Previous studies consider bootstrap values greater than 70 as significant (Landry and Lapointe, 1997). Discussions here are based on that broad rule. The native "control" population clusters together in 84% of the bootstrap samples, indicating a great degree of confidence that this population is discreet from others in the study (figure 11). Other clusters occur less frequently indicating less difference between populations using the 70% threshold for significance.

V. Conclusions:

My results support findings of previous studies regarding the local-scale genetic impact of translocations. Ellsworth et al. (1994a, 1994b) and Leberg et al. (1999) found genetic evidence from translocation in the immediate area around translocation sites for white-tailed deer. However, little impact was seen on a regional scale. As shown in figures 10 and 11, native Marion County control deer have not experienced significant gene flow from translocated individuals placed in the Harris County and Callaway Gardens populations. The latter populations, however, cluster closely with individuals from source populations in Wisconsin.

For example, some clusters are composed of Gardens individuals or Gardens and Harris individuals. This is expected, since both populations are from the same area and have only been intermittently separated by a fence for the past ten years. Because of this, it is likely that the H and G populations share genetic information. Other groups are composed of Harris, Gardens, and Sandhill or Harris, Gardens, Wisconsin individuals. This clustering indicates a similarity between northern deer source populations and the southern deer where they were translocated. Note, however, that there is not a high degree of confidence in these smaller clusters. On the other hand, there is statistical significance (84%) for the cluster containing all Marion County individuals except M7. The M7 individual clustered more closely with a Wisconsin deer, 9W. All remaining sample individuals cluster together in 98% of the bootstrap resamples and include both Wisconsin populations and both populations that received translocated individuals. This indicates that all local deer sampled near the historical translocation event share genetic information similar to populations in Wisconsin where the original source population was

gathered. However, deer in Marion County share little relative genctic similarity indicating no impact from the Harris County translocation event approximately 50 miles away.

Recall also that an original conclusion by Ellsworth et al. (1994a, 1994b) was that southern deer populations exhibited greater genetic variation in mitochondrial DNA than their northern counterparts. This is likely a result of recent glaciation in North America, which allowed older southern populations to persist while their northern counterparts were forced to recolonize more recently. While RAPD PCR used in my study is not as fine-scale as the mtDNA markers of the Ellsworth (1994a, 1994b) studies; recent recolonization would account for the clustering of genetically similar deer in Wisconsin and Sandhill populations. The decreased genetic diversity indicated supports findings by Ellsworth (1994a, 1994b) and lends some credence to the results presented here.

As a final note, it is interesting that the Marion County "native" group clusters most closely with a group from Callaway Gardens (183G, 78G, 72G, 177G). The cluster is not significant, but the Gardens population may be more closely related due to its partial separation from other deer in the area by tall fencing for more than a decade. The partial fencing would then have served as a localized impediment to gene flow. Wisconsin deer released in Harris County would have had greater genetic disturbance from human impact than those in the Gardens. This would lead to more movement of deer and perhaps exchange of genetic information making the Harris deer more similar to those in Wisconsin. The Gardens population, partially isolated, would have maintained a

greater proportion of native genetic information, making them more similar to the Marion deer.

A. Suggestions for Further Study:

A comparison of RAPD data and analysis with other types of molecular population analysis on identical individuals could illuminate the degree of accuracy sacrificed when using RAPD PCR. Regarding this study in particular, a larger sample size of native populations would allow greater confidence in the stated conclusions.

Due to potential problems using RAPD analysis in this study, further investigation of alternative methods seems prudent. Microsatellite primers might be used successfully as markers for population genetic studies due to high levels of polymorphism and broad distribution. The limitation on their use here is the development of primer sets for specific organisms. However, microsatellite markers are available for white-tailed deer (Dewoody et al., 1995). PCR can also be used to amplify cytochrome-b genes from deer, as was done by Carr and Hughes (1993) to study introgression of genes from mule deer into white tail populations. Although potentially more accurate, these techniques would likely resolve allelic bands unscorable without radioassay techniques. Unless two alleles differ in molecular weight significantly, ethidium bromide will not distinguish between bands. There is the possibility of isolating an allelic system, which varies sufficiently to resolve differences between experimental populations while remaining consistent within populations. However, this possibility would be unknown until late in the experimental process after a large commitment of time and funds.

B. Management Implications:

This study indicates that evidence for success or failure of a translocation strategy for whitetail deer can be detected years after the event at least on a local scale. However, the results and methods used here have some application in a broader sense. Lomolino and Channell (1995) indicated a need for extensive planning to account for population genetic structure as part of any translocation strategy. In today's political and economic environment, an in-depth study is often not feasible. By using RAPD PCR as an expedient method to estimate genetic structure, source and destination populations can be matched as closely as possible. In this way, translocation can be made more successful and location of translocation or stocking events can be optimized. More closely matched populations will disrupt natural biogeography less and allow greater survivability of translocated animals which may be better suited to a particular ecosystem type. For example, large healthy deer were translocated from Wisconsin to Louisiana scrubland in the hopes that larger Louisiana deer would result. In fact, the opposite was true. When faced with different climate and food availability, the translocated deer produced smaller, less healthy, offspring than the native Louisiana populations (Day et al., 1999).

Subsequent monitoring using RAPD PCR or some similar method can also be used to monitor translocation success or failure inexpensively for many years. Although RAPD PCR is not as powerful as alternative techniques, it is significantly less time-consuming and expensive. In light of limited funding for rare species conservation, this technique may be the only affordable alternative.

This approach may also be useful in addressing questions of conservation law. For example, a recent dispute between the governor of Maine and National Marine

Fisheries Service revolves around molecular analysis. A question arose regarding whether populations of endangered salmon were native populations deserving of special protection or the result of 130 years of stocking efforts. Based on analysis of scale samples, the data showed that stocking was unsuccessful and native populations persist. There is speculation by scientists involved, about ambiguity and interpretation of the data. At stake is the level of protection the fishes will receive in Maine (Young, 2001). RAPD PCR could resolve the question of genetic similarity in an expedient manner and allow subsequent stocking to reflect the genetics of natural salmon populations.

One can envision a situation in which sampling in the field and RAPD analysis precedes each translocation for a rare species recovery, perhaps while the translocated species are held in captivity. The results of analysis could then be compared with previous RAPD surveys for the recipient population to determine the best location to release the new individuals for minimal genetic disruption. The expense and logistics involved would be far less than a more comprehensive population genetic analysis and could be conducted by scientists in the field without sending samples away for lengthy analysis. This approach could be readily adapted to a variety of situations.

References:

Armstrong, J., A.Gibbs, R. Peakall, and G. Weiller. 1996. RAPDistance version 1.04. Australian National University, Canberra, Australia.

Black, W.C.IV. 1993. PCR with arbitrary primers: approach with care. Insect Molecular Biology 2(1): 1-6.

Caetano-Anolles, G., B.J. Bassam, and P.M. Gresshoff. 1991. DNA amplification fingerprinting using very short arbitrary oligonucleotide primers. Bio/Technology 9: 553-557.

Carr, S.M. and G.A. Hughes. 1993. Direction of introgressive hybridization between species of North American deer (*Odocoileus*) as inferred from mitochondrial-cytochrome-b sequences. Journal of Mammology 74(2): 331-342.

Comincini, S., M. Sironi, C. Bandi, C. Giunta, M. Rubin, and F. Fontana. 1996. RAPD analysis of systematic relationships among the Cervidae. Heredity 76: 215-221.

Day, J.W., B. Zielinski, and M.K. Johnson. Preliminary antler performance of translocated northern white-tailed deer in southeastern Louisiana. 22nd Annual Meeting Southeast Deer Study Group; 1999 February 14-17. Fayetteville, Arkansas.

DeWoody, J.A., R.L. Honeycutt, and L.C. Skow. 1995. Microsatelite markers in whitetailed deer. The Journal of Heredity 86(4): 317-319.

Doremus, H. 1999. Restoring endangered species: The importance of being wild. Harvard Environmental Law Review 23: 1-91.

Ellsworth, D.L., R.L. Honeycutt, N.J. Silvy, M.H.Smith, J.W. Bickham, and W.D.Klimstra. 1994a. White-tailed deer restoration in the southeastern United States: Evaluating genetic variation. Journal of Wildlife Management 58(4): 686-697.

Ellsworth, D.L., R.L. Honeycutt, N.J. Silvy, J.W. Bickham, W.D. Klimstra. 1994b. Historical biogeography and contemporary patterns of mitochondrial DNA in White-tailed Deer from the Southeastern United States. Evolution 48: 122-136.

Engel, S.R., R.A. Linn, J.F. Taylor, and S.K. Davis. 1996. Conservation of microsatelite loci across species of artiodactyls: Implications for population studies. Journal of Mammology 77(2): 504-518.

Falk, D.A. and P. Olwell. 1992. Scientific and policy considerations in restoration and reintroduction of endangered species. Rhodora 94(879): 287-315.

Felsenstein, J. 1985b. Confidence limits on phylogenies: an approach using the bootstrap. Evolution 39: 783-791.

Felsenstein, J. 2000. PHYLIP (Phylogeny inference package version 3.6a. Distributed by the author. Department of Genetics, University of Washington, Seattle.

Griffith, B., J.M. Scott, J.W. Carpenter and C. Reed. 1989. Translocation as a species conservation tool: status and strategy. Science 245: 477-489.

Hadrys, H., M. Balick and B. Schierwater. 1992. Amplifications of random amplified polymorphic DNA (RAPD) in molecular ecology. Molecular Ecology 1: 55-63.

Jeffries, L. 1975. The history of the Georgia deer stocking program, 1928-1974. Fed. Aid Wildl. Restor., Atlanta. 39pp.

Jukes, T. H. and C. R. Cantor. 1969. Evolution of protein molecules. pp. 21-132 in Mammalian Protein Metabolism, ed. H. N. Munro. Academic Press, New York.

Klein-Lankhorst, R.M., A. Vermunt, R. Weide, T. Liharska, and P. Zabel. 1991. Isolation of molecular markers for tomato (*L. esculentum*) using random amplified polymorphic DNA (RAPD). Theor. Appl. Genet. 83: 108-114.

Kostia, S., J. Palo, and S.-L. Varvio. 1996. DNA sequences of RAPD fragments in artiodactyls. Genome 39: 456-458.

Landry, P.A., and F.J. Lapointe. 1997. RAPD problems in phylogenetics. The Norwegian Academy Science and Letters, Zoologica Scripta 25: 283-290.

Leberg, P.L., P.W. Stangel, H.O. Hillestad, R.L. Marchinton, and M.H. Smith. 1994. Genetic structure of reintroduced wild turkey and white-tailed deer populations. Journal of Wildlife Management 58(4): 698-711.

Leberg, P.L. and D.L. Ellsworth. 1999. Further evaluation of the genetic consequences of translocations on southeastern white-tailed deer populations. Journal of Wildlife Management 63(1): 327-334.

Lomolino, M.V. and R. Channell. 1995. Splendid isolation: Patterns of geographic range collapse in endangered mammals. Journal of Mammology 76(2): 335-347.

Lubow, B.C. 1996. Optimal translocation strategies for enhancing stochastic metapopulation viability. Ecological Applications 6(4): 1268-1280.

Lynch, M. and G. Milligan. 1994. Analysis of population genetic structure with RAPD markers. Molecular Ecology 3: 91-99.

Manlove, M.N., J.C. Avise, H.O. Hillestad, P.R. Ramsey, M.H. Smith, and D.O. Straney. 1975. Starch gel electrophoresis for the study of population genetics in white-tailed deer. Proc. Southeast. Assoc. Game and Fish Comm. 29: 392-403.

Marchinton, R.L. and D.H. Hirth. 1984. Chapter 6. Behavior. In: Halls, L.K. (ed.) WhiteTailed Deer Ecology and Management, Stackpole Books. Harrisburg, PA. Pp.129-169.

Meunier, J.-R., and P.A.D. Grimont. 1993. Factors affecting reproducibility of random amplified polymorphic DNA fingerprinting. Res. Microbiol. 144: 373-379.

Mistretta, O. 1994. Genetics of species re-introductions: Applications of genetic analysis. Biodiversity and Conservation 3: 184-190.

Nei, M. and W. H. Li. 1979. Mathematical model for studying genetic variation in terms of restriction endonucleases. Proceedings of the National Academy of Sciences, USA 76: 5269-5273.

Nelson, M.E. 1993. Natal dispersal and gene flow in white-tailed deer in northeastern Minnesota. Journal of Mammology 74(2): 316-322.

Noble, R.E. 1966. Mississippi's deer herd; past and present. Mississippi Game and Fish 29: 14-15.

Page, R.D.M. 2000. TreeView version 1.6.1. Glasgow, UK: University of Glasgow.

Perez, T., J. Albornoz, and A. Dominguez. 1998. An evaluation of RAPD fragment reproducibility and nature. Molecular Ecology 7:1347-1357.

Qaigen Inc. 1999. Taq PCR Handbook. 38pp.

Scribner, K.T., M.H. Smith, and R.K. Chesser. 1997. Spatial and temporal variability of microgeographic genetic structure in white-tailed deer. Journal of Mammology 78(3): 744-755.

Smith, M.H., H.O. Hillestad, R. Baccus and M.N. Manlove. 1984. Chapter 5. Population genetics. In: Halls, L.K. (ed.) White-Tailed Deer Ecology and Management, Stackpole Books. Harrisburg, PA. Pp.119-129.

Swofford, D. 2000. PAUP 4.0. Sinauer and Associates, Inc. Sunderland, MA.

Tear, T.H. and D. Forester. 1992. Role of social theory in reintroduction planning: A case study of the Arabian Oryx in Oman. Society and Natural Resources 5: 359-374.

Welsh, J. and M. McClelland. 1990. Fingerprinting genomes using PCR with arbitrary primers. Nucleic Acids Research 18(24): 7213-7218.

Williams, J.G.K., A.R. Kubelik, K.J. Livak, J.A. Rafalski, and S.V. Tingey. 1990. DNA plymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Research 18(22): 6531-6535.

Williams, J.G.K., M.K. Hanafey, J.A. Rafalski, and S.V. Tingey. 1993. Genetic analysis using random amplified polymorphic DNA markers. Methods in Enzymology 218: 704-740.

Wing, E.S. 1965. Early history. In: R.F. Harlow and F.K. Jones Jr. (eds.) The white-tailed deer in Florida. Florida Game and Fresh Water Fish Commission Technical Bulletin 9, Tallahassee, FL. Pp. 5-12.

Young, S. Listing Maine salmon wrong, King tells panel. Bangor Daily News. 2001 June 13.

Appendix I

Band Scoring Data:

Deer ID	OPC -8															
Band Size	2050	1550	1530	1500	1420	1410	1400	1380	1300	1200	1100	1010	990	890	850	450
M1	1	0	0	0	1	0	1	0	0	0	0	1	0	1	0	0
M2	1	0	0	0	0	1	1	0	0	0	0	1	0	1	0	0
M3	1	0	0	0	0	1	1	0	0	0	0	1	0	1	0	0
M4	1	0	0	0	0	1	1	0	0	0	0	1	0	1	0	0
M5	1	0	0	0	0	1	1	0	0	0	0	1	0	1	0	0
M6	1	0	0	0	0	1	1	0	0	0	0	1	0	1	0	0
M7	1	0	0	0	0	1	1	0	0	0	0	1	0	1	0	0
Deer ID	OPC-8															
Band Size	2050	1550	1530	1500	1420	1410	1400	1380	1300	1200	1100	1010	990	890	850	450
22H	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
62H	1	0	0	0	0	0	1	0	0	0	1	1	1	0	0	0
76H	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
21H	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
70H	1	0	0	0	0	0	1	0	0	1	0	1	0	0	0	0
60H	1	0	0	0	1	1	1	0	0	0	0	1	0	0	0	0
84H	1	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1
65H	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
48H	1	0	0	0	0	0	1	0	0	0	0	1	0	0	0	0
59H	0	0	0	0	0	0	1	0	0	0	0	1	0	0	0	1
58H	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
66H	1	0	0	0	1	0	1	0	0	0	0	1	0	0	0	0
64H	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1
61H	1	0	0	0	0	1	1	0	0	1	1	1	0	0	0	0
Deer ID	OPC-8															
Band Size	2050	1550	1530	1500	1420	1410	1400	1380	1300	1200	1100	1010	990	890	850	450
72G	1	0	0	0	0	0	1	0	0	0	0	1	0	0	0	0
78G	0	0	0	0	0	0	1	0	0	0	0	1	0	0	1	0
183G	1	0	0	0	0	0	1	0	0	0	0	1	0	0	1	0
94G	1	0	0	0	0	0	1	0	0	0	0	1	0	0	0	0
177G	1	0	0	0	0	0	1	0	0	0	0	1	0	0	0	0
83G	1	0	0	0	1	0	1	0	0	0	0	1	0	0	0	0
171G	1	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1
200G	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
170G	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
82G	1	0	0	0	0	0	1	0	0	0	0	1	0	0	0	0
Deer ID	OPC-8															
Band Size	2050	1550	1530	1500	1420	1410	1400	1380	1300	1200	1100	1010	990	890	850	450
1W	1	0	0	0	0	1	1	0	0	0	0	1	0	0	0	0

2W	1	0	0	0	0	0	1	0	0	0	0	1	0	0	0	0
3W	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
4W	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
6W	1	0	0	0	0	0	1	1	0	1	1	1	0	0	0	0
8W	0	0	0	0	0	0	1	0	1	1	0	1	0	0	0	0
9W	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
11W	1	0	0	0	0	1	1	0	1	0	0	1	0	0	0	0
12W	1	0	0	0	0	1	0	1	0	0	1	1	1	0	0	0
13W	0	0	0	0	0	0	1	0	0	0	0	1	0	0	0	0
14W	1	0	0	0	0	0	1	0	0	0	0	1	0	0	0	1
15W	1	0	0	0	0	0	1	0	0	0	1	1	0	0	0	0
16W	1	0	0	0	0	0	1	0	0	0	1	1	0	0	0	0
Deer ID	OPC-8															
Band Size	2050	1550	1530	1500	1420	1410	1400	1380	1300	1200	1100	1010	990	890	850	450
11S	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
14S	1	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0
12S	1	0	0	0	0	0	1	0	0	0	1	0	1	0	0	0
17S	1	0	0	0	0	0	1	0	0	0	0	1	0	0	0	0
15S	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1
26S	1	0	0	0	0	0	1	0	0	0	0	1	0	0	0	0
25S	1	0	0	0	0	0	1	0	0	0	0	1	0	0	0	0
24S	1	0	0	0	0	0	1	0	0	0	0	1	1	0	0	0
23S	1	0	0	0	1	0	1	0	0	0	1	1	1	0	0	0
22S	1	0	0	0	0	0	0	1	0	1	0	1	1	0	0	0

Deer ID	**OPC -9**														
Band Size	2400	2300	1900	1600	1500	1450	1400	1350	1300	1200	1100	990	910	810	690
M1	1	1	0	0	1	0	1	0	1	0	1	0	1	1	1
M2	1	1	0	0	1	0	1	0	1	1	1	0	1	1	1
M3	1	1	1	0	1	0	1	0	1	1	1	0	1	1	1
M4	1	1	0	0	1	0	1	0	1	1	1	0	1	1	1
M5	1	1	0	0	1	0	1	0	1	1	1	0	1	1	1
M6	1	1	1	0	1	0	1	0	1	1	1	0	1	1	1
M7	1	1	0	0	1	0	0	0	0	1	1	0	0	0	1
Deer ID	OPC-9														
Band Size	2400	2300	1900	1600	1500	1450	1400	1350	1300	1200	1100	990	910	810	690
22H	0	0	0	0	0	0	0	0	0	0	1	1	0	0	1
62H	1	1	1	1	1	0	0	0	1	1	1	0	0	0	1
76H	1	1	1	0	1	1	1	0	1	1	1	0	0	0	1
21H	1	1	1	1	1	0	0	0	0	1	1	0	0	0	1
70H	1	1	1	0	1	1	0	0	0	0	1	0	0	0	1
60H	1	1	0	0	0	0	1	0	0	1	1	0	0	0	1
84H	1	1	0	1	1	0	0	0	0	0	1	0	0	0	1
65H	1	1	0	0	1	0	0	0	0	0	1	0	0	0	1

48H	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0
59H	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1
58H	1	1	0	0	1	0	0	0	0	0	1	0	0	0	1
66H	1	1	0	0	1	0	0	0	0	0	1	0	0	0	1
64H	1	1	0	0	0	0	0	0	0	0	1	0	0	0	0
61H	1	1	0	0	1	0	0	0	0	0	1	0	0	0	1
Deer ID	OPC-9														
Band Size	2400	2300	1900	1600	1500	1450	1400	1350	1300	1200	1100	990	910	810	690
72G	1	1	1	1	1	0	1	0	1	1	1	0	1	1	1
78G	0	0	0	0	0	0	1	0	0	0	0	0	0	1	0
183G	1	1	0	1	1	0	1	0	0	0	1	0	1	1	1
94G	0	1	1	0	1	0	0	1	1	1	1	0	0	0	1
177G	1	1	1	1	1	0	1	0	0	0	1	0	0	0	1
83G	1	1	1	0	1	0	0	1	0	1	1	0	1	0	1
171G	1	1	0	0	0	1	0	0	0	0	1	0	0	0	1
200G	1	1	1	0	1	0	0	0	0	0	1	0	0	0	1
170G	1	0	1	0	1	1	1	0	0	1	0	0	0	0	1
82G	1	1	1	0	0	0	1	0	1	1	1	0	0	0	1
Deer ID	OPC-9														
Band Size	2400	2300	1900	1600	1500	1450	1400	1350	1300	1200	1100	990	910	810	690
1W	0	1	1	0	0	1	0	1	1	1	1	0	0	0	1
2W	1	1	0	0	0	0	0	0	0	0	1	0	0	0	1
3W	1	1	1	0	1	0	0	0	0	1	1	0	0	0	1
4W	0	0	1	0	0	1	0	0	0	0	1	0	0	0	1
6W	1	1	0	0	1	0	1	0	0	0	1	0	0	0	1
8W	1	1	0	0	0	0	0	0	0	0	1	0	0	1	1
9W	1	1	0	0	0	0	0	0	0	0	1	0	0	0	1
11W	1	1	0	0	0	0	1	0	1	1	1	0	0	0	1
12W	1	1	0	0	0	0	1	0	0	1	1	0	0	0	1
13W	1	0	0	0	0	1	1	0	0	0	1	0	0	0	1
14W	1	1	1	0	1	0	1	0	0	1	1	0	1	1	1
15W	1	1	1	0	1	0	1	1	0	1	1	0	0	0	1
16W	1	1	1	0	1	1	1	0	0	0	1	0	1	1	1
Deer ID	OPC-9														
Band Size	2400	2300	1900	1600	1500	1450	1400	1350	1300	1200	1100	990	910	810	690
11S	1	1	0	0	1	0	1	0	0	0	1	0	0	0	0
14S	1	1	0	0	0	0	1	0	1	1	1	0	0	0	1
12S	1	1	0	0	0	0	1	1	1	0	1	0	0	0	1
17S	1	1	1	0	1	1	0	0	1	0	1	0	0	0	1
15S	1	1	0	0	1	1	1	0	0	0	1	0	0	0	1
26S	1	1	1	0	1	0	0	0	1	1	1	0	0	0	1
25S	1	1	0	0	1	0	1	0	0	0	1	0	1	0	1
24S	1	1	0	0	1	1	1	0	1	1	1	0	0	0	1
23S	1	1	1	0	1	0	1	0	0	0	1	1	0	0	1
22S	1	1	0	0	0	1	0	1	1	0	1	0	0	0	1

Deer ID	OPT-4										
Band Size	1100	1050	1000	925	900	750	720	675	600	590	580
M1	1	0	0	1	0	0	1	0	1	0	0
M2	1	0	0	1	0	0	1	0	1	0	0
M3	1	0	0	1	0	0	1	0	1	0	0
M4	1	0	0	1	0	0	1	0	1	0	0
M5	1	0	0	1	0	0	1	0	1	0	0
M6	1	0	0	1	0	0	1	0	1	0	0
M7	1	0	1	1	0	1	1	0	1	1	0
Deer ID	OPT-4										
Band Size	1100	1050	1000	925	900	750	720	675	600	590	580
22H	1	0	1	0	0	1	1	1	1	0	0
62H	1	0	1	1	0	1	1	1	1	0	0
76H	1	0	1	0	0	1	1	1	1	0	1
21H	1	0	1	1	0	1	1	1	1	1	0
70H	1	0	1	1	0	1	1	1	1	0	1
60H	1	0	1	1	0	1	1	1	1	0	0
84H	1	0	1	1	0	1	1	1	1	1	0
65H	1	0	1	0	0	1	1	1	1	1	0
48H	1	0	1	0	0	1	1	1	1	0	0
59H	1	1	1	0	0	1	1	1	1	1	0
58H	1	0	1	1	1	1	1	0	1	0	0
66H	1	0	1	1	0	1	1	1	1	1	0
64H	1	0	1	1	0	1	1	1	1	0	0
61H	1	0	1	0	0	1	1	0	1	0	0
Deer ID	OPT-4										
Band Size	1100	1050	1000	925	900	750	720	675	600	590	580
72G	1	0	0	0	0	0	1	1	1	1	0
78G	1	0	0	1	0	0	1	1	1	0	0
183G	1	0	1	1	0	0	1	1	1	1	0
94G	1	0	0	0	0	0	1	0	1	1	0
177G	1	0	0	0	0	0	0	1	1	0	0
83G	1	0	1	1	0	1	1	1	1	1	0
171G	1	0	1	1	0	1	1	0	1	0	0
200G	1	0	1	1	0	1	1	1	1	0	0
170G	1	0	1	1	0	1	1	1	1	0	0
82G	1	0	1	1	0	1	1	1	1	1	0
Deer ID	OPT-4										
Band Size	1100	1050	1000	925		750	720	675	600	590	580
1W	1	0	1	1	0	0	1	1	1	0	1
2W	1	0	1	1	0	1	1	1	1	0	1
3W	1	0	1	1	0	1	1	0	1	1	0
4W	1	0	1	1	0	0	1	1	1	1	0
6W	1	0	1	1	0	1	1	1	1	1	1
8W	1	0	1	1	0	1	1	0	1	1	0
9W	1	0	1	1	0	0	1	0	1	1	0

11W	1	0	0	1	0	1	1	1	1	1	0
12W	1	0	0	0	0	1	1	1	1	1	0
13W	0	0	0	0	0	0	1	1	1	1	0
14W	1	0	1	1	0	1	1	1	1	1	0
15W	1	0	1	1	0	0	1	1	1	1	0
16W	1	0	0	0	0	0	1	0	1	1	0
Deer ID	OPT-4										
Band Size	1100	1050	1000	925	900	750	720	675	600	590	580
11S	1	0	1	0	0	1	1	1	1	0	0
14S	1	0	1	0	0	1	1	1	1	0	1
12S	1	0	1	1	1	1	1	1	1	1	0
17S	1	0	1	1	1	1	1	1	1	0	0
15S	1	0	1	1	0	1	1	1	1	1	0
26S	1	0	1	1	0	1	1	1	1	1	0
25S	1	0	1	1	0	1	1	1	1	1	0
24S	1	0	1	0	0	1	1	1	1	0	0
23S	1	0	1	0	0	1	1	0	1	0	0
22S	1	0	1	0	0	0	1	0	1	0	1

Deer ID	**OPC -6**															
Band Size	3000	2500	1900	1550	1500	1490	1480	1470	1400	1390	1000	910	900	890	800	750	70
M1	0	0	0	0	0	0	0	0	1	1	0	0	1	0	0	0	
M2	0	0	0	0	0	0	0	0	1	1	0	0	1	1	0	0	
M3	0	0	0	0	0	0	0	0	1	1	0	0	1	1	0	0	
M4	0	0	0	0	0	0	0	0	1	1	0	0	1	1	0	0	
M5	0	0	0	0	0	0	0	0	1	0	0	0	1	1	0	0	
M6	0	0	0	0	0	0	0	0	1	1	0	0	1	1	0	0	
M7	0	0	1	0	0	1	0	0	1	1	0	0	1	0	1	0	
Deer ID	OPC-6																
Band Size	3000	2500	1900	1550	1500	1490	1480	1470	1400	1390	1000	910	900	890	800	750	70
22H	0	0	0	0	1	1	0	0	0	1	0	1	1	0	0	0	
62H	1	0	1	0	0	1	0	0	1	1	0	0	0	0	1	0	
76H	0	1	0	0	0	0	0	0	1	1	0	0	0	0	0	0	
21H	0	1	0	0	0	1	0	0	1	1	0	1	0	0	0	0	
70H	1	1	1	0	0	1	0	0	1	1	0	0	0	1	0	0	
60H	0	1	1	0	0	1	0	0	1	1	0	0	0	0	0	0	
84H	1	1	1	0	0	1	0	0	1	1	0	1	1	1	0	0	
65H	0	0	0	0	0	1	0	0	1	1	0	0	0	1	0	0	
48H	1	0	0	0	0	0	0	0	1	1	0	0	0	1	0	0	
59H	0	1	0	0	0	1	0	0	1	1	0	1	0	1	0	0	
58H	1	1	0	0	0	0	0	0	1	0	0	0	0	1	0	0	
66H	0	1	0	0	0	0	0	0	1	1	0	0	0	1	0	0	
64H	0	0	0	0	0	1	0	0	1	1	1	0	0	0	0	0	

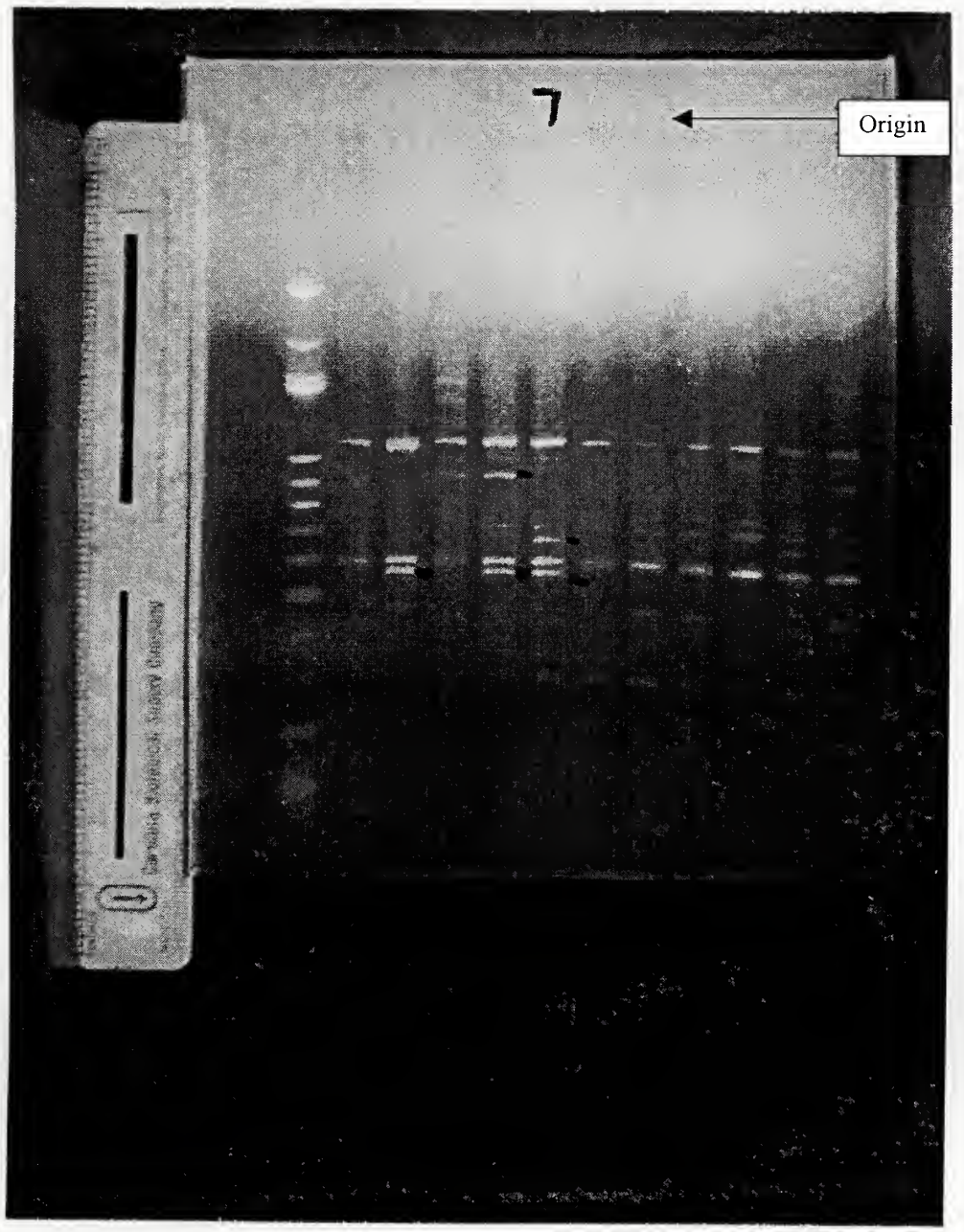
61H	0	1	0	0	0	1	0	0	1	1	0	0	0	0	0	0	
Deer ID	OPC-6																
Band Size	3000	2500	1900	1550	1500	1490	1480	1470	1400	1390	1000	910	900	890	800	750	70
72G	0	0	0	0	0	1	0	0	1	1	0	1	0	1	1	1	
78G	0	0	0	0	0	0	0	0	1	0	0	0	0	1	0	1	
183G	0	0	0	0	0	1	0	0	1	1	0	0	1	1	1	1	
94G	0	1	0	0	0	1	0	0	1	1	0	0	0	1	0	0	
177G	0	0	0	0	0	1	0	0	1	1	0	1	1	1	0	1	
83G	0	1	0	0	0	1	0	0	1	1	0	1	1	1	1	1	
171G	0	0	0	0	0	1	0	0	1	1	0	0	0	1	0	1	
200G	1	1	1	0	0	0	0	0	1	1	0	0	0	1	1	0	
170G	1	0	0	0	0	1	0	0	1	1	0	0	0	1	0	1	
82G	0	1	0	0	0	1	0	0	1	1	0	0	1	1	0	1	
Deer ID	OPC-6																
Band Size	3000	2500	1900	1550	1500	1490	1480	1470	1400	1390	1000	910	900	890	800	750	70
1W	0	0	0	0	0	0	0	0	1	1	0	0	0	1	1	0	
2W	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	
3W	0	1	0	0	0	1	0	0	1	1	0	1	0	0	0	0	
4W	0	0	0	0	0	1	0	0	1	1	0	1	0	1	0	0	
6W	0	0	0	0	1	0	0	0	1	1	0	0	0	0	1	0	
8W	1	1	1	0	0	1	0	0	0	1	0	0	0	0	0	0	
9W	0	0	1	0	0	0	0	0	1	1	0	0	0	0	1	0	
11W	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	
12W	0	1	1	1	0	1	0	0	0	1	0	0	0	1	0	0	
13W	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
14W	0	0	1	0	0	1	0	0	1	1	0	0	0	1	0	0	
15W	0	0	1	0	0	1	0	0	1	1	0	0	0	1	0	0	
16W	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0	0	
Deer ID	OPC-6																
Band Size	3000	2500	1900	1550	1500	1490	1480	1470	1400	1390	1000	910	900	890	800	750	70
11S	0	0	0	0	0	1	0	0	1	1	0	0	1	1	0	0	
14S	0	0	0	0	0	0	1	0	1	1	0	0	0	1	0	0	
12S	0	1	0	0	0	1	0	0	0	1	0	0	0	1	0	0	
17S	1	0	0	0	0	1	0	0	0	1	0	0	0	1	0	0	
15S	0	1	1	0	1	1	0	0	1	1	0	0	0	0	1	0	
26S	1	0	1	0	1	1	0	0	1	1	0	1	0	0	0	1	
25S	0	0	1	1	1	1	0	0	0	1	0	0	0	0	1	0	
24S	0	0	0	0	0	0	0	0	1	1	0	0	0	0	1	0	
23S	0	0	0	0	0	1	0	0	0	1	0	0	0	1	1	0	
22S	0	0	0	0	0	1	0	0	0	1	0	0	0	1	1	0	

Figure 12
Gel scoring data for all analyzed DNA fragments.

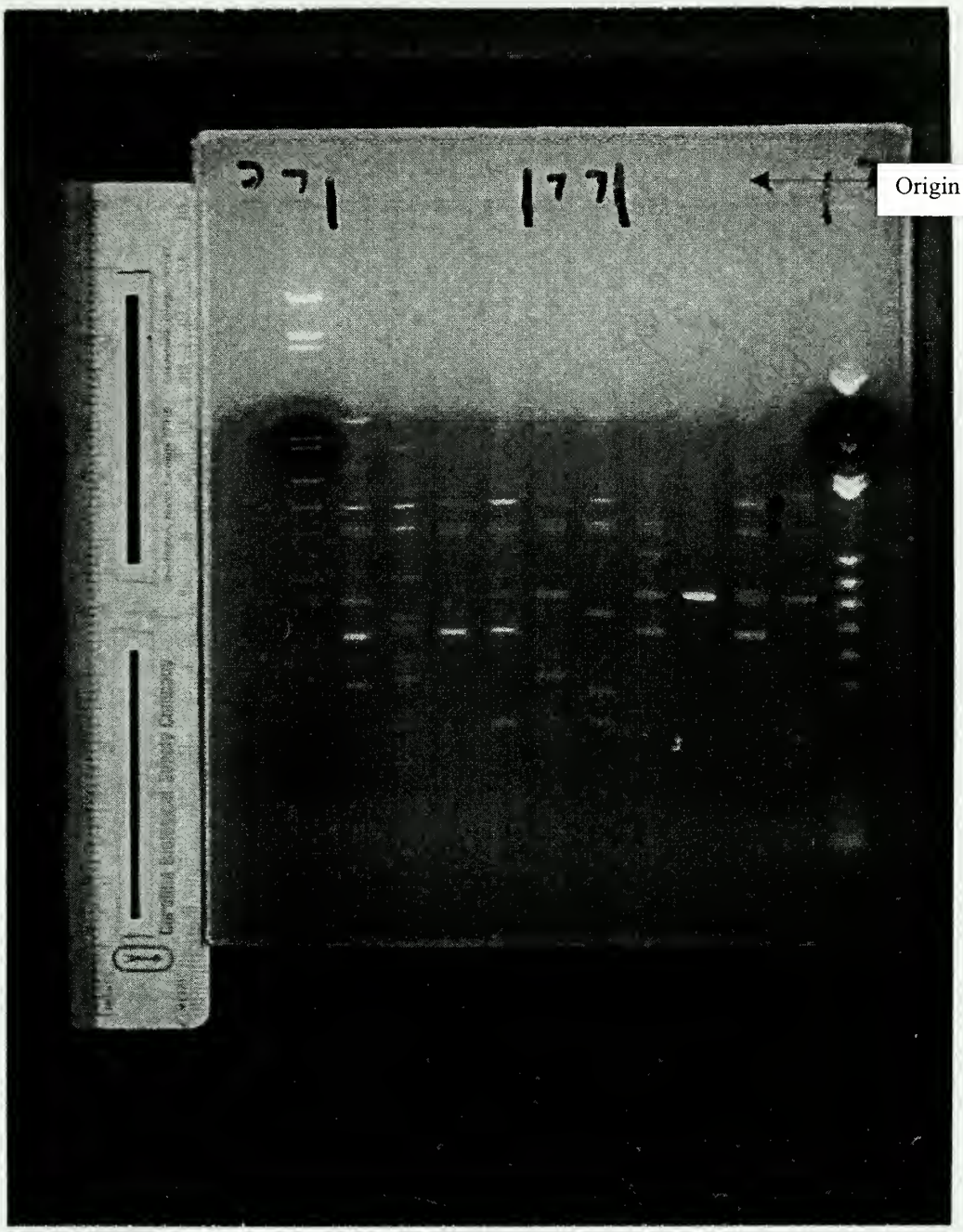
Appendix II

Sample RAPD PCR Gel Images:

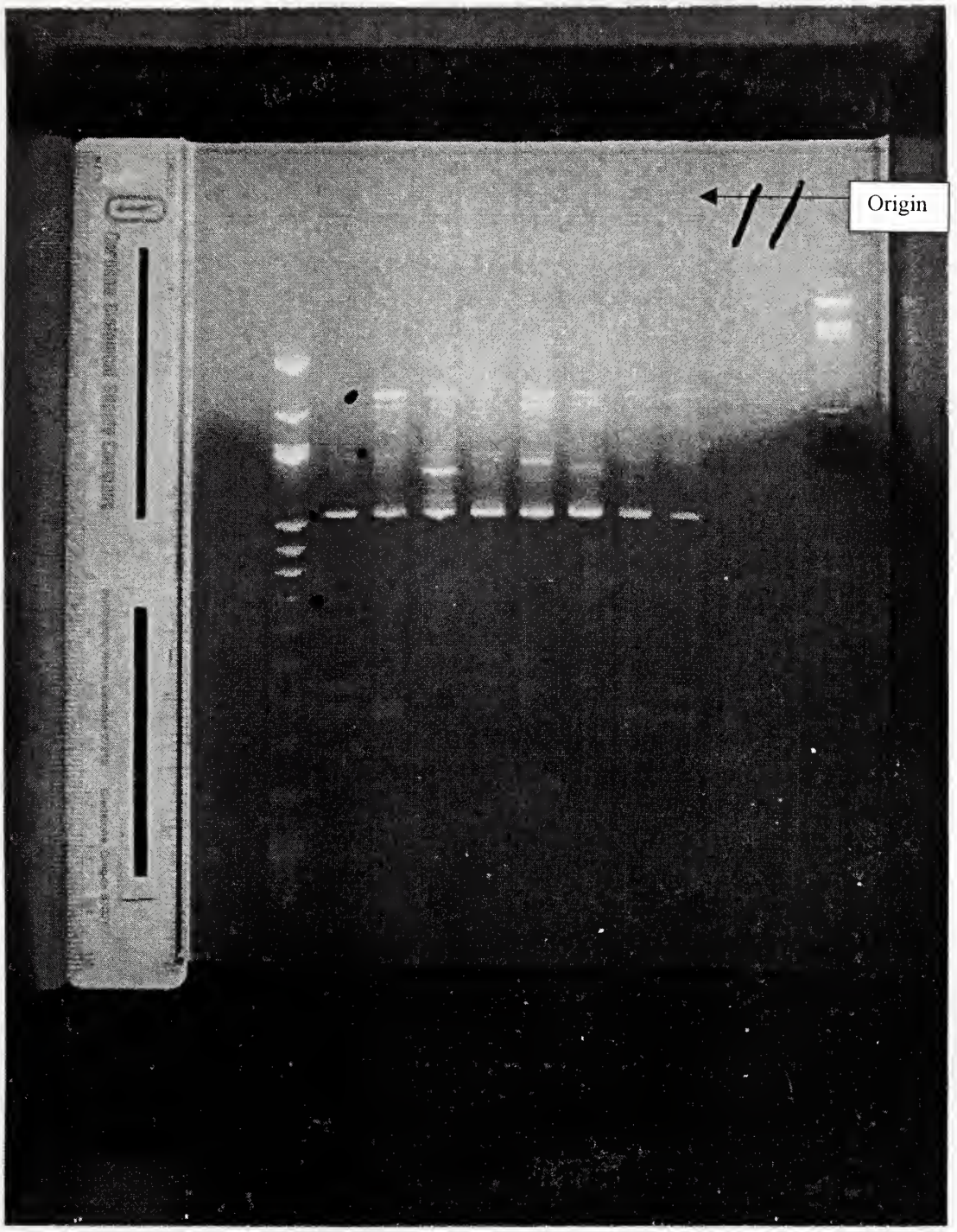
All gels were photographed using a Polaroid™ camera equipped with a red filter. Film was exposed for from 6-8 seconds using an f-stop of 16. Please contact the author for all gels used for analysis.



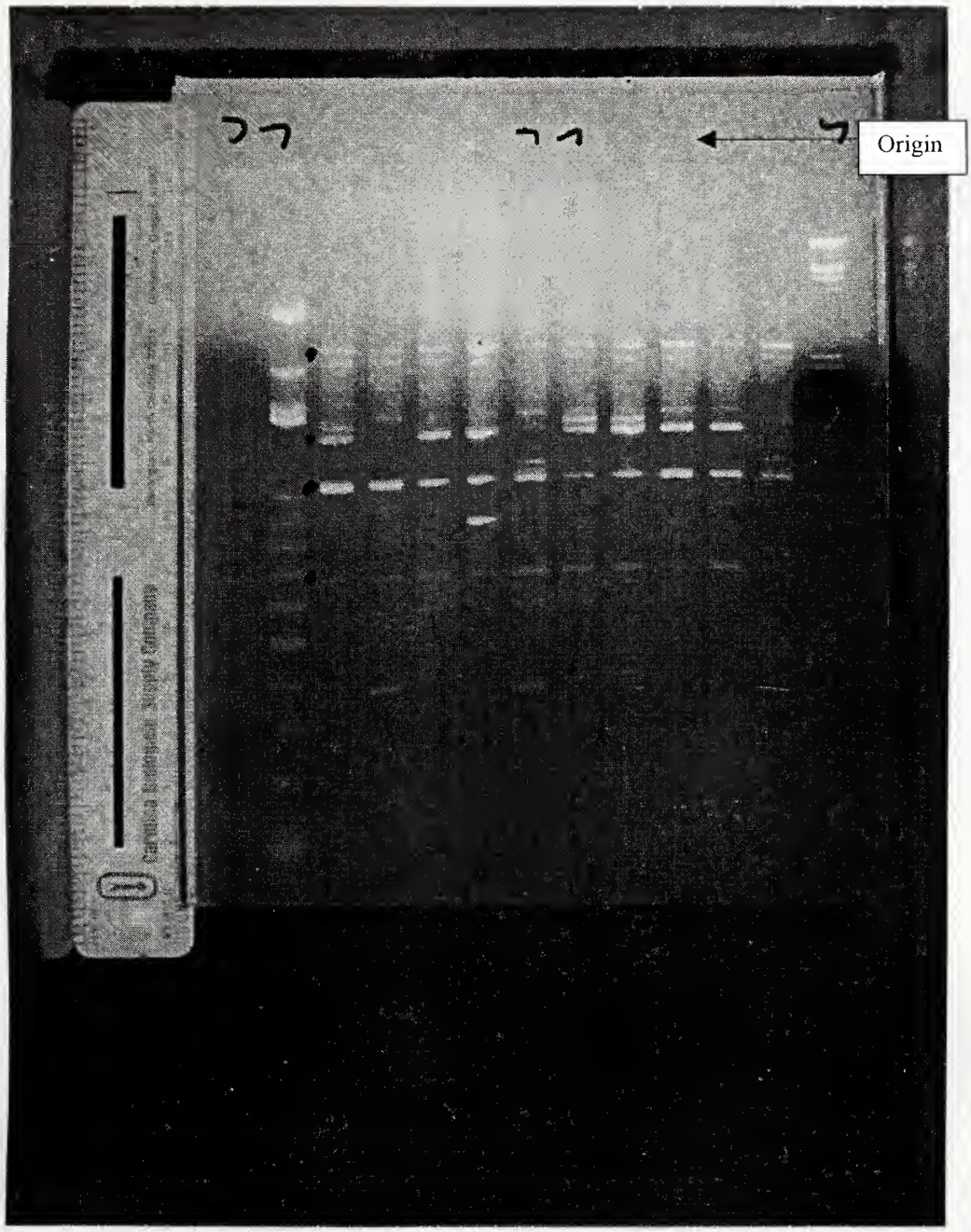
OPC-6 Harris



OPC-6 Sandhill



OPC-9 Harris



OPC-9 Sandhill 2

Appendix III

Gels, Buffers, Protocols:

EDTA Stock Solution

186.1g disodium ethylenediaminetetraacetate · H_2O (EDTA)

800.0mL deionized H_2O

Stir

Adjust pH to 8.0 using NaOH pellets (approx. 20g NaOH)

Filter or autoclave

TBE Buffer Solution 5X

54.0g Tris

27.5g Boric Acid

20.0mL 0.5M EDTA (pH 8.0)

Bring to 1L volume using deionized H_2O

Dilute to 1X concentration using deionized H_2O

1.4% Agarose Gel

2.8g Agarose

200.0mL 1X TBE buffer

10.0μL Ethidium Bromide

Melt using conventional microwave, pour, and insert well comb

0.7% Agarose Gel

0.35g	Agarose
50.0mL	1X TBE buffer
2.5µL	Ethidium Bromide

PCR Reactants

12.5µL	Qiagen, Inc. Master Mix
10.5µL	deionized H_2O
1.0µL	Operon Technologies, Inc. Primer
1.0µL	template sample DNA

Thermal Profile

Melting Phase	94 C	1min.
Annealing Phase	40 C	1min.
Elongation Phase	72 C	2min.
X 45 cycles		
Hold Phase	0 C	10-20 hr.

DNA Isolation Profile

1. Each tissue sample was placed in a sterile 1.5ml microcentrifuge tube. Tissue was lysed using 20µl of Proteinase K and 180µl of proprietary buffer ATL added to each tube. Samples were incubated at 55°C in a shaking water bath overnight.
2. After lysis, samples were treated with 4µl of RNase to remove any residual RNA, which could interfere with PCR.
3. 200µl of proprietary buffer (AL) was added to each sample and incubated for 10min. at 70°C on an MJ Research PTC-100 thermal cycler.
4. 200µl of absolute ethyl alcohol was added to each tube.
5. The entire mixture was then pipetted into a DNeasy™ spin column and centrifuged for 1min. Column flow through was discarded.
6. The column was placed in a new collection tube and 500µl of proprietary buffer (AW1) was added and centrifuged for 1min. Flow through was discarded.
7. Step 6 was repeated using proprietary buffer (AW2) and centrifuged for 3min.
8. Isolation columns were placed in final collection tubes and 200µl of proprietary buffer (AE) was placed in each tube. Tubes were centrifuged for 1min. This last step was repeated and the collection tube now containing eluted DNA was labeled and stored at -20°C.